

**EFFECT OF PROBIOTIC CHEWING GUM ON CARIES SUSCEPTIBILITY IN
ORTHODONTIC PATIENTS: A RANDOMISED CONTROLLED TRIAL**

Adèle Bronkhorst

A research report submitted to the Faculty of Health Sciences, University of the
Witwatersrand, in partial fulfillment of the requirements for the degree of
Master of Dentistry in the branch of Orthodontics

Johannesburg, 2011

DECLARATION

I, Adèle Bronkhorst, declare that this research report is my own work. It is being submitted, in partial fulfillment of the requirements for the degree of Master of Dentistry in the branch of Orthodontics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Adèle Bronkhorst

17th Day of June, 2011

DEDICATION

This research is dedicated to my family, friends and colleagues, for their support, patience and love, without which none of this would have been possible.

ABSTRACT

Introduction: Treatment with fixed orthodontic appliances leads to the prolonged accumulation of dental plaque. Patients are at risk because they may develop gingival inflammation and enamel demineralisation during treatment. Several studies suggest that chewing probiotic gum improves the oral microbial ecology. The effect in orthodontic patients has not been reported. **Aim:** To evaluate the effect of a probiotic chewing gum on saliva flow, plaque and gingival indices and mutans streptococci and lactobacilli in orthodontic patients. **Methods:** 27 orthodontic patients with fixed appliances completed a double-blind, randomised cross-over study over a period of 16 weeks. The DMFS, sucrose and fiber consumption was assessed. Following a washout period of 28 days either a probiotic gum containing 10^8 CFU/gum *Lactobacillus reuteri* ATCC 55730 and PTA 5289 or a placebo were chewed for 10 minutes twice daily for 28 days. Plaque and gingival indices and saliva flow were measured initially and after chewing gum. Saliva was cultured and the number of mutans streptococci and lactobacilli determined. The results were analysed using the Student's t-test and ANOVA. **Results:** The placebo gum reduced saliva flow significantly ($p=0.032$) while this effect was not significant after patients chewed the probiotic gum. There was a decrease in the plaque and gingival indices and salivary microorganisms, and an increase saliva flow in 26% of the patients after they chewed the probiotic gum. **Conclusion:** Chewing sugar-free probiotic gum may reduce the risk of developing white spot lesions and gingival inflammation in susceptible orthodontic patients.

ACKNOWLEDGEMENTS

My sincerest gratitude to my supervisor, Professor M.M. Coogan (Department of Clinical Microbiology and Infectious Diseases, Division of Oral Microbiology, University of the Witwatersrand) whose tireless guidance during the experimental phase and advice in preparation of this research report was of immense value.

Many thanks to Professor P. Fatti (School of Statistics & Actuarial Science, University of the Witwatersrand) for his assistance with the statistical analyses.

Many thanks to Professor W.G. Evans (Head, Department of Orthodontics, University of the Witwatersrand) for his advice during preparation of this report.

Many thanks to Professor A Volchansky (Department of Orthodontics, University of the Witwatersrand) for proof reading of the document.

I wish to express my sincere thanks to:

Messrs BioGaia AB for the donation of the probiotic and placebo chewing gums used.

Messrs Colgate-Palmolive (Pty) Limited for the donation of toothbrushes and toothpaste used by the participants during the study.

The South African Dental Association for funding the cost of the agar plates.

Ms E Dibakwane for her assistance in the microbiological laboratory.

The participants and their primary care givers for their willingness to take part in the study and adhering with the requirements of the study.

TABLE OF CONTENTS

	PAGE
DECLARATION	ii
DEDICATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vii
LIST OF FIGURES	xi
LIST OF TABLES	xii
CHAPTER 1: INTRODUCTION	1
1.1 FACTORS INVOLVED IN THE CARIES PROCESS	1
1.1.1 Oral hygiene and dental caries	2
1.1.2 Diet and dental caries	3
1.1.3 Saliva and dental caries	5
1.1.4 Mutans streptococci and dental caries	7
1.1.5 <i>Lactobacilli</i> and dental caries	8
1.2 WHITE SPOTS LESIONS AND ORTHODONTIC TREATMENT	8
1.3 PREVENTION OF WHITE SPOT LESIONS	10
1.3.1 Fluoride and the prevention of white spot lesions	10
1.3.2 Diet and the prevention of white spot lesions	12
1.3.3 Oral hygiene and the prevention of white spot lesions	13
1.3.4 Saliva and the prevention of white spot lesions	13
1.3.5 Probiotics and the prevention of white spot lesions	14

1.3.6 Chewing gum and the prevention of white spot lesions	17
1.4 THE PRESENT STUDY	20
1.4.1 Aim of the study	20
CHAPTER 2: MATERIALS AND METHODS	22
2.1 ETHICS CLEARANCE	22
2.2 SELECTION OF PARTICIPANTS	22
2.3 CHEWING GUM	23
2.4 STUDY DESIGN	23
2.5 CLINICAL EXAMINATION	26
2.5.1 Decayed, missing and filled permanent tooth surfaces (DMFS)	26
2.5.2 Plaque Index (PI)	27
2.5.3 Gingival Index (GI)	28
2.6 SALIVA ANALYSIS	29
2.6.1 Stimulated salivary flow rate	29
2.6.2 Microbiological analysis of saliva	29
2.7 DIET ANALYSIS	30
2.8 STATISTICAL ANALYSIS	31
CHAPTER 3: RESULTS	33
3.1 DECAYED, MISSING AND FILLED PERMANENT TOOTH SURFACES (DMFS)	33
3.2 DIET	33

3.3 SALIVA FLOW AND MUTANS STREPTOCOCCI AND <i>LACTOBACILLI</i>	34
3.3.1 Saliva Flow	34
3.3.2 Mutans streptococci in saliva	34
3.3.3 <i>Lactobacilli</i> in saliva	34
3.4 PLAQUE AND GINGIVAL INDICES	35
3.5 OVERALL RESULTS	36
 CHAPTER 4: DISCUSSION	 40
4.1 DMFS	40
4.2 DIET	41
4.3 SALIVA	42
4.3.1 Saliva flow	42
4.3.2 Mutans streptococci and <i>Lactobacilli</i> in saliva	43
4.4 PLAQUE AND GINGIVAL INDICES	46
4.5 RESPONSE TO THE PROBIOTIC GUM	47
4.6 MULTIFACTORIAL NATURE OF DENTAL CARIES	48
4.7 PREVENTION OF WHITE SPOT LESIONS IN ORTHODONTIC PATIENTS	49
 CHAPTER 5: CONCLUSIONS	 51

APPENDICES

APPENDIX A: List of abbreviations used in text and tables	52
APPENDIX B: Ethics clearance certificate	53
APPENDIX C: Forms for participants	54
Information sheet for parents and legal guardians	55
Information sheet for participants	57
Informed consent for parents/legal guardians	59
APPENDIX D: Analyses recording forms	60
DMFS	61
Gingival index	62
Plaque index	63
Diet analysis	64
REFERENCES	65

LIST OF FIGURES

Figure 2.1 Outline of the crossover study design. Periods 1 and 3 indicate the washout periods while periods 2 and 4 indicate the periods when a gum was used 25

LIST OF TABLES

Table 3.1	The mean number of decayed, missing and filled surfaces and the dietary intake at the initial visit	37
Table 3.2	Plaque index, gingival index, saliva flow, <i>Lactobacilli</i> and mutans streptococci in saliva at the initial visit (Initial) and the difference between the results at the initial visit and after chewing Probiotic (Probiotic) and Placebo (Placebo) gum for 4 weeks.	38
Table 3.3	The number of patients with a low, moderate and high plaque indices and no inflammation or mild, moderate or severe gingival inflammation at the initial visit and after chewing Probiotic and Placebo gum for four weeks	39

CHAPTER 1: INTRODUCTION

In recent years orthodontics has played an important role in dentistry and this trend is likely to continue in the future. A survey of the long-term effects of orthodontic treatment revealed that the majority of individuals who had undergone orthodontic treatment felt they benefitted and were satisfied with the result (Al-Omiri & Alhaija, 2006). Although many patients experience dramatic improvement in their dental and facial aesthetics, occlusal function and oral health, treatment with fixed orthodontic appliances has disadvantages because it may be associated with gingival inflammation and enamel demineralisation (Morrow, Woode & Speechley, 1992). Gingivitis is reversible while enamel demineralisation is usually irreversible (Øgaard, Rølla, Arends, *et al.*1988).

White spot lesions are the first clinical signs of enamel demineralisation and dental caries (Mizrahi, 1982). More research has been undertaken on the caries process than the development of white spot lesions. Because these terms indicate different degrees of the same process the terms will be used interchangeably in this report.

1.1 FACTORS INVOLVED IN THE CARIES PROCESS

The aetiology of dental caries is due to intricate events within microbial colonies that may become established on intact enamel surfaces. The caries process is complex and is influenced by several factors including the susceptibility of the teeth, the regular intake of fermentable dietary carbohydrates, the quantity and quality of saliva and the presence of large numbers of cariogenic microorganisms in the mouth. The convoluted interactions between

these multiple factors are complex and ultimately determine whether demineralisation will occur (Fejerskov & Thylstrup, 1994).

1.1.1 Oral hygiene and dental caries

Fixed appliances alter the oral environment by increasing the number of sites on the teeth that retain plaque. Thus it becomes more difficult for patients to clean their teeth and predisposes them to stagnant plaque accumulation particularly around brackets and bands and adjacent sites along the gingival margin (Pender, 1986). Furthermore, appliances may restrict the ability of the tongue to remove food from these stagnant areas (Yip, Wong & Hägg, 2009).

Studies in the Scandinavian countries show that poor oral hygiene and high sugar intake are both related to dental caries, although sugar has the stronger relationship (Kleemola-Kujala & Räsänen, 1982; Stecksén-Blicks, Arvidsson & Holm, 1985). Oral hygiene practices and the age at which dental care commences could explain differences in the levels of caries. However there were little regional differences in sugars intake frequency (Moynihan & Petersen, 2004). Furthermore there is a significant association between poor compliance with home care preventive measures and the formation of white spot lesions. Statistical analyses have shown significant differences in the compliance levels of males and females and amongst varying age groups (Geiger, Gorelick, Gwinnett, *et al.* 1988).

1.1.2 Diet and dental caries

There is a wealth of evidence implicating dietary sugars in the aetiology of dental caries and several observational and intervention human studies have been undertaken (Stecksén-Blicks, Arvidsson & Holm, 1985; Touger-Decker & Van Loveren, 2003). These include human plaque studies that monitor the pH of plaque *in situ* following ingestion of a test substance. Other studies investigated the effect of the consumption of experimental diets on enamel bovine slabs fitted into removable oral appliances worn for a particular period. Thereafter the level of demineralisation was measured. In addition there have been studies on animals and the incubation of oral bacteria and dietary substrates *in vitro* (Moynihan & Petersen, 2004). Information from all the studies provides an overall picture of the cariogenic potential of dietary carbohydrates. The strength of the evidence linking sugars in the aetiology of dental caries comes from the diversity of the studies rather than the power of any one study. It is worth noting that some of the earlier epidemiological studies were undertaken in communities that did not have the benefit of exposure to fluoride (Moynihan & Petersen, 2004).

The evidence shows that sugars are undeniably the most important and investigated dietary factor implicated in the development of dental caries. In this report, the term ‘sugars’ indicates all mono- and disaccharides while the term ‘sugar’ only refers to sucrose; the term ‘free sugars’ refers to all mono- and disaccharides added to foods by manufacturer, cook or consumer, plus sugars naturally present in honey, fruit juices and syrups. The term ‘fermentable carbohydrate’ refers to free sugars, glucose polymers, fermentable oligo-saccharides and highly refined starches (Moynihan & Petersen, 2004).

The frequency of the intake of sugars is an important aetiological factor implicated in caries development (Moynihan & Petersen, 2004). Cleaton-Jones, Richardson, Winter, *et al.* (1984) reported noteworthy associations between the frequency and amount of sugar intake in a number of South African ethnic groups. There is also evidence to show that these variables influence the occurrence of dental caries. However, the indication for one particular parameter is not strong (Moynihan, 2002).

Many of the earlier studies investigating the relationship between sugars and dental caries focused on sucrose, which at that time was the main sugar added to the diet. However, the modern diets of industrialised countries contain a mixture of carbohydrates including sucrose, glucose, lactose, fructose, glucose syrups, high fructose corn syrups, other synthetic oligosaccharides and highly processed starches that are fermentable in the mouth. Oral bacteria metabolise all mono- and disaccharides to produce acid and animal studies have shown no clear evidence, with the exception of lactose that the cariogenicity of monosaccharides differ. However, early plaque pH studies have shown plaque bacteria produce less acid from lactose compared with other sugars (Moynihan & Petersen, 2004).

The cariogenicity of sugary food is related to its oral retention. Therefore the longer it takes for a food to clear the mouth the longer a low pH will be maintained. However, the adhesiveness or 'stickiness' of a food is not necessarily related to either oral retention or cariogenic potential. There is evidence that both the amount and frequency of consumption of high sugar drinks with low stickiness and limited oral retention are none the less associated with an increased risk of dental caries (Moynihan & Petersen, 2004).

Starch constitutes a heterogeneous food group and it varies in botanical origin. It may be highly refined or consumed in its natural state but is most frequently consumed in a cooked form. All these factors should be considered when assessing the potential and relative cariogenicity of starches. Some researchers argue that cooked and processed starches enter into the caries process because starches are broken down by salivary amylase releasing glucose, maltose and maltotriose and that these are metabolised by oral bacteria to produce acids (Moynihan & Petersen, 2004). Enamel slab experiments in humans have shown that raw starch is not associated with demineralisation and that cooked starch is about one-third to one-half as cariogenic as sucrose. However, mixtures of starch and sucrose would seem to be potentially more cariogenic than starch alone although the level of caries that developed was related to the sucrose concentration in the mix (Moynihan & Petersen, 2004).

1.1.3 Saliva and dental caries

Saliva is one of the most important modifying factors in the caries process. During orthodontic treatment with fixed appliances, the highest incidence of demineralisation occurs in the upper anterior teeth, a site with little exposure to saliva (Gorelick, Geiger & Gwinnett, 1982). This implies a correlation between resistance to demineralisation and salivary flow rate.

Salivary flow rate, pH and buffer capacity contribute to the intra-oral acidogenic balance (Newbrun, 1989). The pH of saliva and its buffer capacity determine the efficacy of neutralisation of the acid produced locally in the oral cavity. An intraoral environment with a low pH may favour colonisation by aciduric mutans streptococci (Svanberg, 1980). A high

salivary pH has a good buffering capacity and does not favour the growth of mutans streptococci, which in turn leads to a lower caries incidence (Edgar, Dawes & O'Mullane, 2004). Furthermore the salivary buffer capacity correlates directly with caries incidence (Newbrun, 1989). Fortunately the flow rate, pH and buffering capacity of saliva all increase during fixed appliance therapy. This helps to counteract the acid challenge and prevent demineralisation (Chang, Walsh & Freer, 1997). Unfortunately during orthodontic treatment with fixed appliances, oral hygiene procedures are often not adequate and negate the positive effect of the increased salivary parameters.

Under normal conditions saliva at a pH of 7 is super-saturated with calcium and phosphate which favours the deposition of calcium. If a demineralised lesion is formed it usually remineralise; although this is a slow process that competes with those factors that cause demineralisation. If the pH in the mouth remains high enough for a sufficient length of time, then complete remineralisation of enamel may occur. However, if the acid challenge is too great, diffusion of calcium and phosphate ions out of enamel dominates and the enamel becomes more porous until finally a carious lesion forms. Thus caries occurs when demineralisation exceeds remineralisation. The rate of demineralisation is affected by the concentrations of hydrogen and fluoride ions. Fluoride inhibits the demineralisation process and the frequency with which the plaque pH falls below the critical pH value without subsequent remineralisation. Thus caries occurs when demineralisation exceeds remineralisation (Moynihan & Petersen, 2004).

1.1.4 Mutans streptococci and dental caries

Mutans streptococci are important bacteria in the development of dental caries because they readily produce organic acids from dietary sugars and like most aciduric bacteria, can synthesise insoluble extracellular dextrans from dietary sugars, a factor that aids bacterial colonisation of the tooth surface (Moynihan & Petersen, 2004). Growth of these streptococci requires the presence of fermentable monosaccharides. Mutans streptococcal invertase splits sucrose into glucose and fructose, which can be metabolised to produce mainly lactic but also other acids including acetic and formic. The resulting low pH alters the plaque ecology. A low pH in plaque is ideal for aciduric bacteria such as streptococci and lactobacilli because they are more competitive at a low pH than are those bacteria not associated with dental caries (Moynihan & Petersen, 2004).

Mutans streptococci are regularly isolated from humans and are considered the major cause of dental caries (Foley, Aggarwal & Hatibovic-Kofman, 2002). In some patients an increase in the number of *Streptococcus mutans* in plaque may lead to a higher incidence of enamel demineralisation (Jordan & LeBlanc, 2002). A disproportionate increase of *S. mutans* in patients with orthodontic appliances has been reported in several studies (Corbett, Brown, Keene, *et al.* 1981; Chang, Walsh & Freer, 1999; Beyth, Redlich, Harari, *et al.* 2003; Sari & Birinci, 2007). This suggests that a marked reduction of oral mutans streptococci maintained through orthodontic treatment should reduce the risk of developing white spot lesions and dental caries.

1.1.5 Lactobacilli and dental caries

The lactobacilli are aciduric and acidogenic bacteria that prefer hard surfaces for growth. They appear to have a relatively low affinity for natural tooth surfaces. However, the presence of orthodontic appliances dramatically increases the numbers of these organisms in the mouth (Van Houte, Gibbons & Pulkkinen, 1972; Chang, *et al.* 1999). After the removal of orthodontic appliances the *Lactobacillus* salivary count return to original levels (Sakamaki & Bahn, 1968). Lactobacilli are linked to the advancement of carious lesions into the dentine because they cannot form plaque on the tooth on their own and are dependent on extracellular polysaccharides produced by other oral organisms, mainly streptococci, for colonisation (Russell & Ahmed, 1978). The presence of lactobacilli in large numbers indicates that the necessary conditions for producing white spot lesions and dental caries exist in the mouth (Chang, *et al.* 1999).

1.2 WHITE SPOTS LESIONS AND ORTHODONTIC TREATMENT

The white appearance of early enamel caries is an optical phenomenon which is caused by mineral loss in the superficial or subsurface enamel. Enamel crystal dissolution begins with subsurface demineralisation, creating pores between the enamel rods. The modification of the refractive index in the affected area is a result of both surface roughness and loss of surface shine and alterations in internal reflection, all resulting in greater visual enamel opacity, as porous enamel scatters more light than sound enamel (Gorelick, *et al.*, 1982; Øgaard, 1989). The demineralisation process may include the full thickness of the enamel and some of the

dentine prior to actual loss of the relatively hypermineralised surface layer (Sudjalim, Woods & Manton, 2006).

Orthodontic treatment with fixed appliances has been associated with white spot lesions that often occur in otherwise successful cases. The overall prevalence among orthodontic patients varies from 2-96% (Zachrisson & Zachrisson, 1971; Mizrahi, 1982; Mitchell, 1992), depending upon the methods used to assess and score demineralisation, the presence of demineralisation before treatment and the use of fluoride supplements during treatment. Studies have reported an increase in the number of lesions in patients undergoing orthodontic therapy with fixed appliances (Mizrahi, 1982; Geiger, *et al.* 1988). These demineralised areas have been detected within the first month of cementing orthodontic bands (Øgaard, *et al.* 1988) and their subsequent appearance is often related to the duration of treatment (Gorelick, *et al.* 1982).

White spot lesions develop in association with directly-bonded brackets, bands, arch wires, ligatures and other orthodontic devices that lead to prolonged plaque accumulation (Øgaard, *et al.* 1988). In the presence of fermentable carbohydrate, demineralisation of the enamel around the bracket can occur within four weeks. Labial surfaces commonly show more evidence of demineralisation among patients with fixed appliances whereas the palatal surfaces are at risk in patients with removable appliances (Geiger, *et al.* 1992). Enamel lesions have been recorded on all the teeth but are observed most frequently on the cervical and middle third of the buccal surface of the maxillary lateral incisors, the mandibular canines and the first premolars (Gorelick, *et al.* 1982). Although demineralised enamel may remineralise partially after debonding, white enamel lesions are often irreversible (Øgaard, *et al.* 1988). The

presence of these lesions has been confirmed five years after completion of orthodontic treatment and appears to be resistant to remineralisation (Øgaard, 1989).

1.3 PREVENTION OF WHITE SPOT LESIONS

The overall management of white spot lesions involves the prevention of demineralisation and the remineralisation of existing lesions. Studies indicate when preventive measures are followed and maintained throughout the course of orthodontic treatment, the number of white spot lesions may be reduced (Zachrisson, 1975, 1976, 1977; Gorelick, *et al.* 1982). Preventive measures should include the application of antimicrobial agents, for example topical fluorides and antibacterial medicaments, the education of patients regarding oral hygiene and regular professional oral hygiene visits (Zachrisson, 1976). All patients should be given dietary education and be instructed to reduce the amount and frequency of intake of carbohydrates, especially sucrose which is readily fermented by bacteria to produce organic acids which may cause demineralisation.

1.3.1 Fluoride and the prevention of white spot lesions

Fluoride alters the resistance of the teeth to demineralisation as well as the speed at which the enamel surface remineralise following a plaque acid challenge. It affects the tooth post-eruptively in three ways, namely, by affecting demineralisation, remineralisation and bacterial metabolism. Firstly, when fluoride is incorporated into the enamel lattice and/or binds to enamel crystal surfaces it reduces and inhibits demineralisation and replaces the hydroxyl

groups in hydroxyapatite. This is beneficial because fluoroapatite is more stable and more resistant to further acid attacks than hydroxyapatite. Hence, fluoride reduces the susceptibility of the enamel to demineralisation. Secondly, remineralisation of enamel in the presence of fluoride results in porous lesions being reconstructed with fluoroapatite. Lastly, fluoride also affects plaque by inhibiting bacterial metabolism of sugars thus reducing acid production (Moynihan & Petersen, 2004).

Once tooth formation is complete and eruption has begun fluoride acts mainly topically at the enamel surface. The inverse relationship between fluoride in drinking-water and dental caries is well established. Without any dietary modifications, topical fluoride in toothpastes, mouth rinses or varnishes reduce caries in children by 20 - 40%, but does not eliminate the disease. Over 800 controlled trials on the effect of fluoride on dental caries have shown that fluoride is the most effective preventive agent available. Widespread use of fluoride largely accounts for the decline in dental caries that has been observed in developed countries over the past three decades (Moynihan & Petersen, 2004). Although fluoride increases the resistance to demineralisation, it does not remove the major causes of dental caries i.e. dietary sugars and cariogenic bacteria. Fluoride agents have proved to be effective, but their effectiveness depends on patient compliance and the correct use of these agents (Øgaard, *et al.*, 1988).

1.3.2 Diet and the prevention of white spot lesions

From a dietary point of view, the best advice for reducing caries risk is to reduce the amount and frequency of consumption of food and drinks containing sugars and to limit their consumption to mealtimes. It is also advisable to avoid sugars-containing food and drinks an hour before bedtime because salivary buffering capacity and flow is reduced at night (Moynihan, 2002). Plaque pH studies have shown that consuming cheese following a sugary snack virtually abolishes the usual drop in pH associated with sugars consumption. Cheese stimulates salivary secretion and increases plaque calcium concentration. The calcium concentration of dental plaque strongly influences the balance between demineralisation and remineralisation of enamel (Moynihan & Petersen, 2004).

The main reason that fibrous foods protect the teeth is because they stimulate the salivary flow mechanically, clean teeth and encase potentially cariogenic carbohydrates in a non-fermentable layer that is removed when the food is cleared from the mouth (Coogan, MacKeown, Galpin, *et al.* 2008). Other foods that are beneficial include peanuts, hard cheeses and chewing gum that contain gustatory and mechanical stimulants that increase saliva flow. Animal and human studies have also demonstrated that black tea extract increases plaque fluoride concentration and reduces the cariogenicity of a diet rich in sugars because it contains antibacterial fluoride, polyphenols and flavanoids (Moynihan & Petersen, 2004).

1.3.3 Oral hygiene and the prevention of white spot lesions

According to Schwaninger & Vickers-Schwaninger (1979) if the amount of plaque in a patient's mouth can be eliminated or controlled much tissue pathosis could be prevented. Twice daily toothbrushing is one of the most popularly accepted means of food debridement and plaque removal and 91% of responding orthodontists used a basic practice protocol for oral hygiene measures (Derks, Kuijpers-Jagtman, Frencken, *et al.* 2007). With manual brushing, the horizontal brushing techniques (scrub or Bass action) have proved most effective for orthodontic patients. Unfortunately all brushing techniques achieve only limited access to the interdental areas and dental floss and interdental brushes should be used to clean these areas (Schwaninger, *et al.* 1979).

Forsberg, Brattström, Malmberg, *et al.* (1991) recommends that attempts should be made to keep orthodontic appliances as simple as possible to counteract their tendency to increase plaque accumulation. Nevertheless the key point in controlling the risk of hard and soft tissue complications remains the patient's oral hygiene compliance. Motivation is of the utmost importance to ensure and improve compliance. Various preventive measures have been employed as adjuncts to oral hygiene instruction because patient compliance is often lacking (Benham, Campbell & Buschang, 2009).

1.3.4 Saliva and the prevention of white spot lesions

An increase in salivary flow rate removes fermentable substrates and improves the buffer capacity, pH and the antibacterial activities of saliva. This in turn will counteract the acid

challenge and prevent demineralisation (Chang, *et al.* 1999). Studies have shown there is a statistically significant increase in salivary flow rate in orthodontic patients (Ulukapi, Koray & Efes, 1997; Chang, *et al.* 1999) and that an inverse relationship exists between *S. mutans* and stimulated salivary flow (Alamoudi, Farsi, Faris, *et al.* 2004). This implies that the caries risk may be reduced by increasing the salivary flow rate.

The chewing of gum stimulates salivary flow and helps to keep the salivary ducts patent (Curro, 2008). When patients chew sugar-free gum an increase in saliva flow followed by a rise in pH levels is seen. Gray & Ferguson (1996) observed that a sugar-free, low tack chewing gum increased the concentration of buffers in saliva and enhanced saliva flow in orthodontic patients. Chewing sugar-free gum at least three times a day significantly reduces caries irrespective of the type of sugar alcohol it contains (Van Loveren, 2004). These clinical trials show that chewing sugar-free gum protects against dental caries. Sugar-free gum chewing is now accredited by the British Dental Association as helping to prevent dental caries (Moynihan, 2002).

1.3.5 Probiotics and the prevention of white spot lesions

Young adolescents generally have short clinical crowns and teeth that are not fully erupted which may compromise efficient plaque control, making them more prone to develop periodontal complications than adults (Boyd, Leggott, Quinn, *et al.* 1989). In addition, elevated hormonal levels during puberty are associated with an increased degree of gingivitis and gingival hyperplasia (Zachrisson, 1976). For these reasons antimicrobial agents are

frequently administered to these high risk populations. These agents may contain fluorides and antiseptics (Derks, Katsaros, Frencken, *et al.* 2004).

The use of probiotics to improve health is not a new concept. At the beginning of the 20th century the Ukrainian born Noble Prize laureate, Elie Metchnikoff, reported that Bulgarians lived longer than other populations due to their consumption of fermented milk products containing viable bacteria (Twetman & Stecksén-Blicks, 2007). The suggested mode of action was competition with pathogens. Probiotics have a very good risk-benefit ratio making them an ideal treatment modality for various disease processes (Cildir, Germec, Sandalli, *et al.* 2009).

The most common probiotic strains belong to the genera *Lactobacillus* and *Bifidobacterium* (Saxelin, Tynkkynen, Mattila-Sandholm, *et al.* 2005). The ability of *Lactobacillus reuteri* SD 2112 and *Lactobacillus rhamnosus* GC to influence the colonisation of mutans streptococci has been investigated (Haukioja, Loimaranta & Tenovuo, 2008). *L. reuteri* is an obligatory heterofermentative resident of the human gastrointestinal tract. The exact mode of action of *L. reuteri* is unclear but several possibilities have been proposed. Firstly, *L. reuteri* produces anti-microbial substances called reuterin (Talarico, Casas, Chung, *et al.* 1988) and reutericyclin (Gänzle, Hölzel, Walter, *et al.* 2000). These are water soluble, broad-spectrum antimicrobials, effective over a wide pH range and resistant to proteolytic and lipolytic enzymes (el-Ziney & Debevere, 1998). Reuterin has an inhibitory effect on both Gram-positive microorganisms e.g. *Staphylococcus aureus* and Gram-negative microorganisms, e.g. *Escherichia coli* (el-Ziney, van den Tempel, Debevere, *et al.* 1999). Secondly, strains of *L. reuteri* have the ability to block the binding of pathogenic bacteria to host tissue. A recent

study suggests that anti-inflammatory cytokines in the intestinal mucosa inhibit pro-inflammatory cytokines and a similar effect may be the basis for a direct or indirect effect of this bacterium on the gingiva in subjects with gingivitis (Krasse, Carlsson, Dahl, *et al.* 2006). Another possible mode of action is competitive exclusion of pathogens by preventing the adherence of pathogenic bacteria and modifying the protein composition of the salivary pellicle (Haukioja, *et al.* 2008).

Nikawa, Makihira, Fukushima, *et al.* (2004) found that a daily consumption of yogurt products containing *L. reuteri* significantly inhibited the growth and oral carriage of *S. mutans* by up to 80% within a 14 day period. In contrast Montalto, Vastola, Marigo, *et al.* (2004) was not able to demonstrate any effect of probiotic capsules or liquid on salivary *S. mutans* counts after 45 days. Çaglar, Sandalli, Twetman, *et al.* (2005) initially demonstrated that a once daily consumption of yogurt containing *Bifidobacterium* DN-173010 for a period of 14 days led to a significant reduction in the number of the salivary mutans streptococci but no significant reduction in lactobacilli. The following year Çaglar, Cildir, Ergeneli, *et al.* (2006) found a significant reduction of salivary mutans streptococci levels in young adults after ingestion of *L. reuteri* ATCC 55730 through straws or lozenges taken once daily for 21 days. Again there was no significant reduction of salivary lactobacilli. In the same year Krasse, *et al.* (2006) showed that the plaque and gingival indices in test subjects with moderate or severe gingivitis were significantly improved after chewing gum containing *L. reuteri* twice daily for 14 days. In the Krasse, *et al.* (2006) study 65% of the participants were colonised by this micro-organism.

Later in 2007, Çağlar, [Kavaloglu](#), [Kuscu](#), *et al.* demonstrated that groups chewing either a gum containing xylitol or *L. reuteri* ATCC 55730 and *L. reuteri* ATCC PTA 5289 three times daily reduced the level of salivary mutans streptococci significantly. However chewing the probiotic and xylitol gums simultaneously did not further enhance this effect. In 2008, Çağlar, [Kuscu](#), Cildir, *et al.* found that a once a day use of *L. reuteri* ATCC 55730 and *L. reuteri* ATCC PTA 5289 probiotic lozenges in young women reduced salivary mutans streptococci levels after 10 days. More recently Cildir, *et al.* (2009) investigated the effect of once daily probiotic *Bifidobacterium* DN-173010 yogurt consumption in orthodontic patients and demonstrated a significant reduction of salivary mutans streptococci after 14 days, but could not demonstrate a significant change in salivary lactobacilli counts.

There is evidence that the plaque and gingival indices of non-orthodontic participants improved after regular exposure to probiotics. In addition, products containing probiotics reduce salivary mutans streptococci significantly after 21 days of use in non-orthodontic patients. Most studies regarding salivary lactobacilli counts demonstrate no significant reduction of these microorganisms in non-orthodontic patients (Çağlar, *et al.*, 2006; Çağlar, *et al.*, 2007; Çağlar, *et al.*, 2008).

1.3.6 Chewing gum and the prevention of white spot lesions

As mentioned previously the use of sugar-free, low tack chewing gum increases the flow and concentration of buffer ions in the saliva. It also has the potential to promote remineralisation and reduce white spot lesions related to fixed orthodontic appliances (Gray, *et al.* 1996). In a later paper Imfeld (1999) claimed that chewing gum removes food debris and plaque from

teeth, stimulates saliva flow and thereby raises plaque pH, stops demineralisation, promotes remineralisation and reduces gingivitis. He showed that chewing gum *per se* is neither a substitute for, nor an important adjunct to, traditional mechanical oral hygiene. It is a potent stimulant of saliva flow and effectively raises salivary and plaque pH. In addition the chewing of sugar-free gums after meals and snacks can promote remineralisation of early enamel lesions (Burt, 2006). Sugar-substituted chewing gum is non-cariogenic as no active cariostatic properties have been unequivocally established to date.

The advantages of using chewing gum as a carrier for drugs are obvious. It can be used without water and at any time and in any place. Product stability is excellent because the incorporated therapeutic agents are protected from oxygen, light and water. Chewing gum can produce a local effect by direct delivery into the mouth and a systemic effect after the active agents have been swallowed or absorbed through the oral mucosa. The latter method is of particular interest regarding bioavailability, because it avoids the metabolism of the drug in the gastrointestinal tract and the so called liver-first-pass effect, because oral veins drain into the vena cava (Imfeld, 1999).

Many drugs incorporated in chewing gum are lipophilic and bind to the gum base and their release is slow and incomplete. Methods to increase the rate and extent of release include the addition of buffering agents, the coating of drugs with hydrophilic gum, the use of solubilising agents and the incorporation of drugs into the gum by solid dispersion. In contrast drugs with high aqueous solubility are released rapidly. A reduced or sustained release has been attempted by binding these drugs to an ion exchange resin, by reducing particle size and by various coating and embedding techniques (Imfeld, 1999).

The predominant sugar substitutes used in sugar-free chewing gum are polyols, which are low-calorie substances sometimes called “sugar alcohols” because their chemical structure is close to that of sugar and alcohol. The polyols are used to sweeten a number of sugar-free products especially chewing gum for the control of dental caries in the United States. The most frequently used polyols in chewing gum are sorbitol which is a hexatol derived from glucose, and xylitol, a pentatol that occurs widely in nature (Burt, 2006).

Polyols do not promote caries because they are metabolised slowly or not at all by the microorganisms in dental plaque. The most commonly used polyol in the United States is sorbitol. It is 60% as sweet as sucrose and is much less expensive than xylitol. Whilst sorbitol is less effective than xylitol in the control of dental caries, its lower cost makes it appealing to food manufacturers (Burt, 2006).

When the caries potential of sorbitol is considered it has an advantage over sucrose because it can be incorporated into foods and drinks at low concentrations that do not lower the pH of plaque below the critical level where enamel demineralisation occurs. However, sorbitol should be considered a low-cariogenic sweetener rather than a non-cariogenic substitute because consumption of more than two sticks of chewing gum per day increases both acid production in plaque and the number of sorbitol-fermenting microorganisms. Furthermore, when sorbitol is incorporated into soft drink it is fermented slowly by mutans streptococci. Thus cariogenic microorganisms can metabolise sorbitol when their sugar supply is restricted. This form of adaptation has also been demonstrated in animals. Several clinical studies indicated that chewing sugar-free, sorbitol containing gum after meals results in a significant reduction in the formation of dental caries and may promote remineralisation (Burt, 2006).

Stookey (2008) suggests this effect is caused by an increase in saliva flow attributed to the chewing process rather than the effect of sorbitol.

In contrast the antibacterial and caries reduction properties of xylitol are superior to the modest reductions reported with sorbitol gum (Edgar, 1998). Cariogenic microorganisms are not able to metabolise xylitol. Thus xylitol does not decrease plaque pH, enamel demineralisation is prevented, remineralisation is enhanced and plaque bacteria do not proliferate. Salivary mutans streptococci counts drop with consistent use of xylitol-sweetened gum, possibly because replacing sucrose with xylitol “starves” the cariogenic microorganisms. Evidence also suggests that the consistent use of xylitol-sweetened gum reduces plaque accumulation (Mäkinen, Chen, Mäkinen, *et al.* 1996). Chewing any gum stimulates the flow of saliva, which enhances the buffering effect in plaque which is beneficial. Xylitol-sweetened gum is a supplemental practice, not a substitution for a preventive dental program that includes the use of fluoride, consciously applied oral hygiene practices and regular professional examinations (Burt, 2006). More recently Çağlar, *et al.* (2007) demonstrated a significant reduction in salivary mutans streptococci when using probiotic gum.

1.4 THE PRESENT STUDY

1.4.1 Aim of the study

The purpose of this study was to determine whether a sugar-free probiotic chewing gum could reduce plaque and gingival indices, the levels of salivary mutans streptococci and lactobacilli and increase saliva flow in adolescent orthodontic patients wearing fixed orthodontic appliances.

CHAPTER 2: MATERIALS AND METHODS

2.1 ETHICS CLEARANCE

Ethics clearance was granted by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg. The ethics clearance protocol number assigned to the study is M080612.

2.2 SELECTION OF PARTICIPANTS

All participants had a full complement of permanent teeth, excluding the third molars, unless premolar extractions had been performed as part of the orthodontic treatment. On clinical examination all participants presented with healthy teeth, no signs of periodontal disease and were undergoing comprehensive orthodontic treatment with full fixed appliances in both arches which had been in place for a minimum of six months prior to the commencement of the study. Subjects with a history of systemic antibiotic or professional topical fluoride treatment within four weeks prior to baseline measurements and those who habitually used products containing probiotics and/or antibiotics were excluded from the study.

Informed consent was obtained from all subjects prior to their participation in the study. Initially, 35 adolescent orthodontic patients enrolled in the study but poor compliance lead to the exclusion of eight subjects and 27 subjects therefore completed the study. There were 16

girls and 11 boys. The mean age of the girls was 13 years, 7 months with an age range between 12 years, 3 months and 15 years, 11 months. The mean age of the boys was 14 years, 1 month with a range between 13 years, 7 months and 16 years, 3 months.

2.3 CHEWING GUM

Reuteri Probiotic Chewing gum (BioGaia, Stockholm, Sweden) and a placebo gum produced by the same company were used in the study. Each piece of probiotic gum contained 1×10^8 colony forming units (cfu) of live *L. reuteri* strain ATCC 55730 and 1×10^8 cfu live *L. reuteri* strain ATCC PTA 5289, together with isomaltose, gum base, hydrogenated palm oil, hydrogenated cotton seed oil, peppermint flavouring, talc, menthol flavouring, silicon dioxide, magnesium stearate, peppermint oil, sorbitol and sucralose. The placebo had a similar composition but contained no lactobacilli.

2.4 STUDY DESIGN

The investigation was a double-blind study. The clinician, examiners, participants and laboratory assistant did not know which gum was being used by participants during the different stages of the study. Confidentiality was maintained by assigning each participant a number.

A cross-over study design, illustrated in Figure 2.1, was used to increase the power of the study. At the initial visit oral hygiene procedures were standardised by giving the participants oral hygiene instructions and providing them with a medium size Colgate® Extra Clean regular head toothbrush (Colgate-Palmolive (PTY) LTD, Boksburg, Gauteng, South Africa) and Colgate® Maximum Cavity Protection fluoridated toothpaste (Colgate-Palmolive (PTY) LTD, Boksburg, Gauteng, South Africa) that contained 1000ppm monofluorophosphate and 450ppm sodium fluoride. Participants were instructed to refrain from using any other chewing gum, any products containing probiotics, for example, yogurt or multivitamins during the study and to report any use of antibiotics during this period. In addition they were instructed to refrain from eating, drinking and brushing their teeth for at least one hour prior to their next visit which was scheduled 28 days ahead.

During the second visit participants were asked to chew bilaterally on a standardised sterile rubber tube for 10 minutes and to expectorate into a sterile 50ml container. The DMFS (decayed, missing and filled tooth surfaces) was measured and a plaque index (PI) and gingival index (GI) was recorded. Thereafter the subjects were asked to complete a four consecutive day recall diet sheet (Kidd, 2005). These results were to be used as the baseline values for the study.

Participants were randomly divided into two groups and were given a plastic screw top pill container in which the gum had been supplied. An independent member of staff who knew the compositions of the gums marked the containers with either a red or a silver sticker. During the remainder of the study the gums were referred to as either red or silver. They were instructed to chew bilaterally on a new piece of gum for 10 minutes twice daily for 28 days, at

least one hour after brushing their teeth. When they returned for the third visit, a 10 minute saliva sample was collected and the plaque and gingival indices were reassessed.

After the third visit participants were placed on a 28 day washout period and were asked to continue oral hygiene procedures as instructed, use the fluoridated toothpaste provided and to refrain from consuming any chewing gum or probiotic products. On the fourth visit, plaque and bleeding indices were reassessed and a 10 minute saliva sample was collected. The subjects who used the red gum initially were then given the silver gum, while participants who had used the silver gum were given the red gum. After a further 28 day period the participants returned for a final visit, a saliva sample was collected and the plaque and gingival indices were recorded. The identity of the red and silver stickers i.e. whether they indicated the probiotic or placebo gum, was divulged by the independent person only after the statistical analyses of the results were completed.

	Period 1	Period 2	Period 3	Period 4
	28 days	28 days	28 days	28 days
Group 1 (n=13)	wash out	Probiotic Gum	wash out	Placebo Gum
Group 2 (n=14)	wash out	Placebo Gum	wash out	Probiotic Gum
	Probiotic Gum	Twice daily chewing of gum containing <i>L. reuteri</i> strains ATCC 55730 and ATCC PTA 528		
	Placebo Gum	Twice daily chewing of gum with no probiotics		

Figure 2.1 – Outline of the crossover study design. Period 1 and 3 were the washout periods while the probiotic and placebo gum were chewed during Periods 2 and 4.

2.5 CLINICAL EXAMINATION

2.5.1 Decayed, missing and filled permanent tooth surfaces (DMFS)

The DMFS index of Klein, Palmer & Knutson (1938), as described by Wilkens (1999) was used to assess caries experience in the permanent dentition. Teeth removed for orthodontic reasons and lost or restored due to trauma, or were unerupted, congenitally missing or supernumerary teeth and the third molars were not included in the score. The lingual, mesial, distal and occlusal surfaces of the posterior and the labial, lingual, mesial and distal surfaces of the anterior teeth were recorded. The following criteria were used:

D:

- Crown broken down as a result of dental caries
- Caries and restorations present

M:

- Tooth extracted because of dental caries
- Tooth carious, non-restorable and indicated for extraction

F:

- Permanent and temporary fillings
- A tooth with a defective filling but without evidence of dental caries

2.5.2 Plaque Index (PI)

The plaque index (PI) was scored using the Turesky modification of the Quigley-Hein PI (Turesky, Gilmore & Glickman, 1970). Plaque was visualised by asking the patient to chew a disclosing tablet (GUM® Red-Cote® disclosing tablets, Butler, USA). The buccal and lingual surfaces of the Ramfjord teeth (upper right first molar, upper left central incisor, upper left first premolar, lower left first molar, lower right central incisor and lower right first premolar) were examined and recorded. When the first premolars were not present, second premolars were substituted. Lorenz, Bruhn, Brex, *et al.* (2007) showed that the assessment of these teeth is as reliable as full-mouth scoring and simplifies a study without distorting the outcome. The following criteria were used:

- 0 = no plaque
- 1 = discontinuous band of plaque at the gingival margin
- 2 = up to one mm continuous band of plaque at the gingival margin
- 3 = band of plaque wider than one mm but less than one-third of the surface
- 4 = plaque covering one-third or more of the surface, but less than two-thirds of the surface
- 5 = plaque covering two-thirds or more of the surface

The PI was obtained by adding the scores for the buccal and lingual surfaces of the six Ramfjord teeth and dividing it by the number of surfaces examined (twelve). Mean PI scores were interpreted as follow:

- 0.1 – 1.0 = low plaque index
- 1.1 – 2.0 = moderate plaque index
- > 2.0 = high plaque index

2.5.3 Gingival Index (GI)

The gingival index (GI) was determined using the index of Loë & Silness (Wilkins, 1999). The teeth were air dried and examined with the patient in a dental chair provided with a halogen light. The mesial, buccal, distal and lingual gingival areas of the Ramfjord teeth were examined. A probe was pressed on the gingiva to determine the degree of firmness. The probe was then run along the soft tissue wall near the entrance to the gingival sulcus to evaluate bleeding. The areas were scored as follows:

- 0 = normal gingiva
- 1 = mild inflammation – slight change in colour, slight oedema but no bleeding on probing
- 2 = moderate inflammation – redness, oedema and glazing with bleeding on probing
- 3 = severe inflammation – marked redness and oedema with a tendency to spontaneous bleeding, ulceration of the gingivae may also be present

The total score for each tooth was divided by four. The GI was determined by adding the scores for all the teeth and dividing that total by the number of teeth examined (six). The indices range from 0 to 3. Mean GI scores were interpreted as follow:

- < 0.1 = no inflammation
- $0.1 - 1.0$ = mild inflammation
- $1.1 - 2.0$ = moderate inflammation
- $2.1 - 3.0$ = severe inflammation

2.6 SALIVA ANALYSIS

2.6.1 Stimulated salivary flow rate

The volume of saliva collected in the ten minute period of chewing on a sterile rubber tube was measured and the salivary flow rate was recorded.

2.6.2 Microbiological analysis of saliva

Rogosa Agar and Mitis Salivarius Bacitracin Agar was used in the study because they are highly selective and do not support the growth of contaminants (Park, Tanabe, Tinanoff, *et al.* 2006). Mutans Bacitracin Agar (MBA) developed by Gold, Jordan & van Houte (1973) was modified and used for the culture of mutans streptococci. The agar was prepared by

dissolving 90g of Mitis Salivarius Agar (Becton, Dickson & Co., Sparks, Maryland, USA) and 150g sucrose in one litre of distilled water. The mixture was sterilised and 0.001g sterile tellurite and 0.0001g sterile bacitracin added to the agar. Ten-fold serial dilutions of the stimulated saliva samples ranging from 1:1000 to 1:100 000 were prepared in sterile phosphate buffered saline (ph 7) (Cruickshank, Duguid, Marmion, *et al.* 1975a) and 0.1ml of the dilutions were spread on the plates using a sterile bent glass rod.

Rogosa Agar (Merck KGaA, Darmstadt, Germany) which contained 0.002% bromocresolgreen (Difco, Detroit, Michigan, USA) was used for the culture of lactobacilli. Ten-fold serial dilutions of the stimulated saliva samples ranging from 1:100 to 1:10 000 were prepared in phosphate buffered saline (ph 7). 0.1ml samples of the dilutions were spread on Rogosa Agar using a sterile bent glass rod.

The plates were incubated under carbon dioxide in a candle jar for 48 hours at 37°C following the technique described by Coogan, *et al.* (2008). Plates were then examined visually. Colony morphology was used as the primary criterion for the enumeration of mutans streptococci and lactobacilli and the numbers of colonies were counted by two independent examiners. Streptococcal colonies appeared crystalline and firmly attached to the MBA surface and were surrounded by drop of water-soluble glucan (Whiley & Beighton, 1998). The lactobacilli appeared as white, opaque, convex, catalase negative colonies (Holt, Krieg, Sneath, *et al.* 1994) on Rogosa agar. The status of catalase colonies (positive or negative) was identified as described by Cruickshank, *et al.* (1975b). When placing a drop of peroxide on the colony the enzymes in the catalase positive colonies will release oxygen from the

hydrogen peroxide which leads to the production of gas bubbles. In the catalase negative colonies no gas bubbles could be detected.

2.7 DIET ANALYSIS

Participants completed a diet sheet (Kidd, 2005) for four consecutive days. They were requested to keep the sheet with them and ensure that everything they ate and drank over that time was recorded. The daily sucrose and fibre intake was calculated using the tables compiled by Langenhoven, Kruger, Gouws, *et al.* (1991). Frequency of daily sucrose and fibre intake was also determined. Participants were instructed not to consume any foods containing probiotic cultures.

2.8 STATISTICAL ANALYSIS

A comparison of the results from the male and female participants showed there were no significant differences between the plaque and bleeding indices, stimulated salivary flow rate and the levels of mutans streptococci and lactobacilli counts in saliva (male vs. female $p > 0.5$). Therefore the data was analysed as one sample.

The levels of mutans streptococci counts, lactobacilli counts, plaque and gingival indices and salivary flow rate were compared using the Student's t-test. Statistical significance was set at 5% ($p < 0.05$). A cross-over design according to the methods described by Jones & Kenward

(1989) was used to analyse the results. During the first part of the statistical analysis the equality of the carry over effect from the first to the second test period was tested. A carry-over effect was not expected due to the washout period. The second part of the statistical analysis evaluated the equality of the period effect for the same parameters i.e. did the period in which the test was undertaken have an effect on the results. The third part of the statistical analysis tested the treatment effect and determined whether there was a difference between the probiotic and the placebo gum. Patients acted as their own controls due to the cross-over design, thus increasing the power of the experiment to detect a significant treatment effect.

In randomised controlled trials (RCT) random assignment into two or more groups protect against selection bias. The placebo effect was equalised by blinding the subjects to their treatment status. Blinding the clinicians prevents differential treatment of the two groups and blinding the examiners ensures objective grading of the results. Collectively, these factors minimize bias and allow much greater confidence to be placed in the results of the RCT (Huang 2004).

Because of the skewness of the distribution of the microbial colonies, the statistical analyses used logarithmic values for mutans streptococci and lactobacilli counts which gave distributions that were closer to normal. It should be kept in mind that the differences noted were based on a logarithmic scale.

All the results were expressed as the Mean $\pm$ the standard deviation (SD).

CHAPTER 3: RESULTS

3.1 DECAYED, MISSING AND FILLED PERMANENT TOOTH SURFACES (DMFS)

At the initial visit the 27 patients who completed the study had a mean DMFS of 0.85 ± 1.46 with no decayed surfaces or teeth missing due to caries and 0.85 ± 1.46 filled surfaces. The 11 boys who completed the study had a mean DMFS of 0.73 ± 1.56 (Table 3.1). Only three boys had restored surfaces; one had five, the second two and the third one. The DMFS of the 16 girls was 0.94 ± 1.44 made up solely by the filled component. Twenty filled surfaces were found in seven of the girls, one had five, two had four and three had two and one had one filled surface.

3.2 DIET

The mean daily fibre intake was $13.46 \pm 9.23\text{g}$ while the frequency was 3.47 ± 1.20 . The mean daily sugar intake was $61.03 \pm 32.76\text{g}$ and the frequency 2.97 ± 0.98 . The 11 boys had a mean daily fibre intake of $13.47 \pm 8.77\text{g}$ with a frequency of 3.50 ± 0.78 while their mean daily sugar intake was $86.01 \pm 30.87\text{g}$ and the frequency was 3.57 ± 0.90 . The mean daily sugar intake of the 16 girls was $43.86 \pm 21.30\text{g}$ and the frequency was 2.56 ± 0.83 . The mean daily fibre intake of $13.46 \pm 9.82\text{g}$ and the frequency was 3.448 ± 1.449 . The boys had a statistically significantly higher daily sugar intake ($p < 0.0003$) and frequency ($p < 0.006$) than the girls (Table 3.1).

3.3 SALIVA FLOW AND MUTANS STREPTOCOCCI AND LACTOBACILLI

3.3.1 Saliva flow

The stimulated salivary flow rate was reduced in both groups after the 28 day periods of using the chewing gums. This effect was seen to a greater extent after chewing the placebo (-2.75 ± 6.30 ; $p = 0.032$) than after chewing the probiotic gum (-0.32 ± 3.62) (Table 3.2).

3.3.2 Mutans streptococci in saliva

There was a greater reduction in the levels of salivary mutans streptococci when the placebo gum (-0.28 ± 0.73) rather than the probiotic gum (-0.14 ± 1.38) was used. Neither of these differences were significant (Table 3.2).

3.3.3 Lactobacilli in saliva

There was a greater reduction in the levels of lactobacilli in the saliva when participants chewed the placebo rather than the probiotic gum, (-0.19 ± 0.87 and -0.03 ± 1.01 respectively). Neither of these differences were significant (Table 3.2).

3.4 PLAQUE AND GINGIVAL INDICES

The crossover design of the study which incorporated a 28 day washout period prior to each test period eliminated any differences between the carry-over, treatment or period effects of the probiotic or placebo gums on plaque index, saliva flow and the levels of mutans streptococci and lactobacilli in saliva. Therefore these results could be grouped together for the analysis. There was a significant period effect on the gingival index which was generally higher in the first test period than the second ($p = 0.009$).

In the groups chewing the probiotic gum the plaque index was reduced by 0.15 ± 0.60 and the gingival index by 0.06 ± 0.20 while in the placebo group a reduction of the plaque index by 0.05 ± 0.56 and the gingival index by 0.07 ± 0.25 (Table 3.2) was seen. None of these changes were statistically significant. Initially 13 participants had a low plaque index. After using the probiotic gum this increased to 18 and to 17 when participants chewed the placebo gum. A moderate plaque index was initially seen in 11 participants. After chewing the probiotic gum six participants had a moderate score and seven after the placebo gum. Three subjects that had a high plaque index initially, after chewing the probiotic and the placebo gum (Table 3.3).

All the participants in this study exhibited some level of gingival inflammation during the study. Mild inflammation was initially identified in 11 participants and was also seen in 14 participants who chewed the probiotic gum and in 13 participants after using the placebo gum. Moderate inflammation was initially identified in 15 participants, while it was seen in 13

participants who had chewed the probiotic gum and 14 participants after the placebo gum had been used. Only one participant had severe inflammation initially (Table 3.3).

3.5 OVERALL RESULTS

When the results were analysed statistically there were no significant changes in the plaque and gingival indices and the levels of microorganisms in saliva. However, there was a significant reduction in the saliva flow among the subjects who had chewed the placebo gum ($p = 0.032$).

Table 3.1 – The mean number of decayed, missing and filled surfaces and the dietary intake at the initial visit

	Total	Boys	Girls
Number of Subjects	27	11	16
DMFS	0.85 ± 1.46	0.73 ± 1.56	0.94 ± 1.44
Decayed	0	0	0
Missing due to caries	0	0	0
Filled	0.85 ± 1.46	0.73 ± 1.56	0.94 ± 1.44
Diet			
Fibre intake	13.46 ± 9.23	13.47 ± 8.77	13.46 ± 9.82
Fibre frequency	3.47 ± 1.20	3.50 ± 0.78	3.45 ± 1.45
Sugar intake	61.03 ± 32.76	86.01 ± 30.87*	43.86 ± 21.30*
Sugar frequency	2.97 ± 0.98	3.57 ± 0.90 [#]	2.56 ± 0.83 [#]
* $p < 0.0003$; [#] $p < 0.006$			

Table 3.2 – The mean plaque index, gingival index, saliva flow, mutans streptococci and lactobacilli in saliva at the initial visit and the difference between the levels from the washout visit and those recorded after chewing probiotic or placebo gum for 28 days

	After the probiotic gum	After the placebo gum
Mean ± SD		
Number of subjects	27	27
Plaque Index	-0.15 ± 0.60	-0.05 ± 0.56
Gingival Index	-0.06 ± 0.20	-0.07 ± 0.25
Saliva Flow	-0.32 ± 3.62	-2.75 ± 6.30 [†]
Mutans Streptococci	-0.14 ± 1.38	-0.28 ± 0.72
Lactobacilli	-0.03 ± 1.01	-0.19 ± 0.87
[†] p=0.032		

Table 3.3 – The number of patients with low, moderate and high plaque indices and no inflammation or mild, moderate or severe gingival inflammation at the initial visit and after chewing probiotic and placebo gum for 28 days

	Initial visit	After the probiotic gum	After the placebo gum
Plaque Index			
0.1 – 1.0 = low	13	18	17
1.1 – 2.0 = moderate	11	6	7
> 2.0 = high	3	3	3
Gingival Index	Initial visit	After the probiotic gum	After the placebo gum
Level of inflammation			
< 0.1 None	0	0	0
0.1–1.0 Mild	11	14	13
1.1–2.0 Moderate	15	13	14
2.1 – 3.0 Severe	1	0	0

CHAPTER 4: DISCUSSION

When the results were analysed statistically there were no significant changes in the plaque and gingival indices and the levels of microorganisms in saliva. This is probably caused by the large inter-individual differences and is supported by the results obtained by Krasse, *et al.* (2006). Dental decay is a complex, chronic disease and several risk factors have been identified over many years. However, these variables may not explain the development of the disease entirely given the multifactorial characteristics of dental caries and the strong influence exerted by several biological, environmental and genetic factors (Werneck, Mira & Trevilatto, 2010).

4.1 DMFS

The DMFS is used as an indication of a patient's caries experience and future caries risk. In this study 18 participants recorded DMFS scores of zero. A minority within the group had considerable caries experience and therefore should historically be more at risk to develop future caries. For example, Al Mulla, Kharsa, Kjellberg, *et al.* (2009) found that orthodontic patients with an initial DMFS ≥ 5 ran a higher risk of developing caries during treatment. Only three boys and seven girls had restored surfaces. A boy and a girl had a DMFS of five, two girls a DMFS of four, three girls and one boy a DMFS of two while one girl had a DMFS of one. Therefore when applying the rules of Al Mulla, *et al.* (2009) only two participants had a high risk of developing dental caries during treatment.

4.2 DIET

The mean intake and frequency of sucrose consumption of all the participants was a total of 61g per day, consumed in at least three portions. There was a significant difference between the diets of the boys and girls. The mean sucrose intake of the males was 86g and of the females, 44g ($p < 0.0003$) while the frequency was 3.6 and 2.6 times a day respectively ($p < 0.006$). Despite these variations in the dietary intake there were no significant differences in the levels of mutans streptococci and lactobacilli in saliva, and the flow rate of saliva in the boys and the girls. The boys consumed most of their sugar in the form of drinks that would be cleared readily from the mouth. In contrast the girls consumed sucrose in the form of sweets, cakes and biscuits that are retentive and would accumulate readily on the teeth especially around the orthodontic appliances (Touger-Decker & Van Loveren, 2003).

There should be no hesitation in endorsing the advice of Yip, *et al.* (2009) that patients should be educated about the consequences of consuming sugar-containing soft drinks and be given positive personal guidelines to promote general and dental health. For example they should avoid consuming sugar-containing food and drinks an hour before bedtime and limit the frequency of sugar intake to a maximum of four times per day as suggested by Moynihan & Petersen (2004). Orthodontic patients should also be encouraged to consume a diet high in fibre as this cleans the teeth mechanically and stimulates the saliva flow thereby protecting the teeth.

4.3 SALIVA

4.3.1 Saliva flow

In the current study the saliva flow was reduced when participants chewed both the probiotic and the placebo gum. The flow rate, pH and buffering capacity of saliva are the most important modifying factors affecting the demineralisation process. In patients with decreased saliva flow rate, bacterial clearance is reduced. Therefore more bacteria will be present in the saliva and be available to colonise the oral tissues (Dawes, 2008). However only the placebo gum caused a significant reduction in flow with $p = 0.032$ (Table 3.2) while the probiotic gum did not have a significant detrimental effect.

These results differ from previous studies that reported an increase in salivary flow when sorbitol-containing gum was used by normal subjects (Stookey, 2009) and by patients undergoing orthodontic treatment (Gray, *et al.* 1996). Furthermore Imfeld (1999) found that chewing sugar-free gum is a potent stimulant of salivary flow rate. The reasons for these differences are not clear. The probiotic gum contained strains of *L. reuteri* that may have been suspended in chemicals that caused this small change in the flow rate.

Salivary flow rate may also be influenced by a large number of factors, including the degree of hydration of subjects, body position, exposure to light, previous stimulation, circadian and circannual rhythms, gland size and drug use (Dawes, 2008). Matos-Gomes, Katsurayama, Makimoto, *et al.*, (2010) demonstrated in a group of medical students that salivary flow rate

was significantly reduced during final exam times. The significant reduction in salivary flow rate seen in some participants when chewing the placebo gum may have been during exam times but this factor was not accounted for in this study.

Salivary flow rate may furthermore have been influenced by the female sex hormones, specifically oestrogen. Percival, Challocombe, Marsh (1994) put forth oestrogen as the main factor responsible for the suppression of salivary flow in females while Streckfus, Baur, Brown, *et al.*, (1998) confirmed the detrimental effects of oestrogen fluctuation and associated changes in the composition and flow rate of saliva on the oral health of women. Animal research verified that fluctuations in oestrogen levels influence thyroid activity which in turn leads to a decrease in saliva flow and an increase in caries rate (Delman, 1955). In this study neither oestrogen nor thyroid levels were analysed for any of the participants at any given time. Fluctuations in these hormonal levels were not accounted for and these may have been further factors causing the reduction in salivary flow rate seen when chewing the gum. It is recognised that girls of the age group in the sample will have raised oestrogen levels.

The reduction in salivary flow by the chewing gum was an unexpected and undesirable effect because food and bacterial clearance could be reduced and the number of bacteria in saliva could increase, attach and colonise the teeth (Dawes, 2008). If fermentable carbohydrates are consumed this could lead to an increase in the number of saccharolytic bacteria, the production of acids followed by an increased risk of demineralisation and the subsequent development of dental caries.

4.3.2 Mutans streptococci and lactobacilli in saliva

In normal subjects not receiving orthodontic treatment, mutans streptococci counts of $>10^6$ indicate a high risk, $\leq 10^5$ indicates reduced risk while values of $<10^4$ are considered a low risk of developing dental caries (Kidd & Joyston-Bechal, 1987). None of the participants in the present study had counts of less than 10^4 mutans streptococci at the initial examination. This was not unexpected because a marked increase in the number of *S. mutans* counts has been reported in patients with orthodontic appliances (Scheie, Arneberg & Krogstad, 1984; Chang, *et al.* 1999; Beyth, *et al.* 2003; Sari, *et al.* 2007). There was a reduction in the number of mutans streptococci to below 10^4 with three subjects who chewed the probiotic gum while similar results were obtained from two participants who chewed the placebo gum. Thus five subjects benefited from chewing the probiotic or the placebo gum which suggests that the effect of chewing the gum was more beneficial than the inhibitory effect of the probiotic lactobacilli.

The probiotics may not have had an influence because the mutans streptococci counts were lower after subjects chewed both gums and the effect was greater when the subjects chewed the placebo rather than the probiotic gum. However neither of these changes were significant (Table 3.2). This is contrary to the findings of previous workers who reported a significant decrease in mutans streptococci when *L. reuteri* containing products were tested in non-orthodontic participants (Nikawa, *et al.* 2004; Çağlar, *et al.* 2005; Çağlar, *et al.* 2006; Çağlar, *et al.* 2007; Çağlar, *et al.* 2008).

These differences may be explained by the differences in study designs as in the Çaglar, *et al.* 2007. In this study the participants were not wearing orthodontic appliances, were aged between 21 and 24 years, were asked to chew the gum three times a day after meals for a period of three weeks and the study did not have a cross-over design. Participants received one week's supply of gum at the outset and were given the next week's supply at the subsequent weekly recall visits in order to monitor compliance. In the present study participants were wearing orthodontic appliances, chewed the gum twice daily as recommended by the manufacturer and a cross-over study design was used. In addition the four-weekly recall coincided for practical reasons with the participants' usual orthodontic appointment.

The non-significant reduction in the number of lactobacilli supported earlier studies that investigated the effect of *L. reuteri* containing products (Çaglar, *et al.* 2006; Çaglar, *et al.* 2007, Çaglar, *et al.* 2008) and found no significant reduction in normal subjects.

Care should be taken when interpreting these results because many studies report there is an increase in the number of lactobacilli in saliva in patients wearing orthodontic appliances (Van Houte, *et al.* 1972; Chang, *et al.* 1999). Van Houte, *et al.* (1972) attributed this increase to the mechanical retention of food by appliances which provided essential carbohydrates that favour the multiplication of the lactobacilli. Eight participants had levels of lactobacilli below 10^4 initially. After chewing the probiotic gum nine participants achieved this level and 11 participants after using the placebo. This reduction in the levels is beneficial because an increase in lactobacilli contributes to the accumulation of plaque seen in many orthodontic

patients (Kupietzky, Majumdar, Shey, *et al.* 2005). The presence of lactobacilli in large numbers indicates that the conditions necessary for the production of white spot lesions and dental caries exists (Chang, *et al.* 1999). This suggests that levels of lactobacilli in orthodontic patients should be monitored regularly to prevent the development of white spot lesions.

The high levels of saccharolytic bacteria indicate that the orthodontic patients are at greater risk. Therefore, the amount and frequency of the use of probiotic gum, although adequate to reduce mutans streptococci and lactobacilli in non-orthodontic patients, may not have been sufficient to reduce bacterial loads in orthodontic patients where food traps are considerably increased.

Another reason for the slight change in microbial counts could be explained by the fermentation of sorbitol, sucralose and isomaltose that were used as sweeteners in the gums. Sorbitol is metabolised slowly by mutans streptococci while other cariogenic microorganisms can adapt their metabolism to this sugar in animals when their sugar supply is restricted (Burt, 2006). The remaining sugars probably had no effect because sucralose is considered non-cariogenic (Moblely, 2003; Matsukubo & Takazoe, 2006) while a number of serotypes of mutans streptococci do not produce acid from isomaltose (Matsukubo, *et al.* 2006).

4.4 PLAQUE AND GINGIVAL INDICES

The plaque and gingival indices were both reduced when participants chewed the probiotic and the placebo gum. However neither of these effects were statistically significant (Table 3.2). The reduction in the plaque and gingival indices is supported by Krasse, *et al.* (2006) who reported a decrease in gingivitis when non-orthodontic patients chewed a probiotic gum for two weeks. The present study also indicated there was a statistically significant period effect because there was a greater reduction in the gingival index following the first chewing period than the second. This period effect did not influence the validity of the overall comparisons.

Gingivitis is one of the most common oral infections in man and is caused by the accumulation of dental plaque along the gingival margin (Signoretto, Bianchi, Burlacchini, *et al.* 2010). The present study supports this theory. Although gingivitis does not necessarily progress to periodontitis, it always precedes that condition. Therefore early identification and intervention is of utmost importance (Oh, Eber, Wang, 2002) especially in patients with fixed orthodontic appliances who often develop periodontal problems (Demling, Demling, Schwestka-Polly, *et al.* 2010). Thus the use of gum is potentially a valuable preventive measure.

The decrease in the plaque index may also have been influenced by participation in this study and not by chewing gum because participants were informed during their first visit that their plaque indices would be monitored regularly. This may have had an effect on the plaque

indices of one participant which decreased from 4.25 at the initial visit to 3.00, 2.08 and 2.33 at the subsequent visits. This improvement was sustained after participation in the study even though the orthodontist who was treating this patient had been unable to motivate him at the start of treatment. The levels of mutans streptococci and lactobacilli were also reduced which suggests that an improvement in the oral hygiene status had a positive influence on the plaque and gingival indices. The decrease in the number of lactobacilli and mutans streptococci may also have reduced the risk of developing white spot lesions in this patient. These findings are supported by Zachrisson (1976) who emphasized that orthodontists should not miss the unique opportunity to improve the patient's oral hygiene and dental awareness by undertaking regular oral hygiene instruction and motivation which would increase the long-term benefits of orthodontic treatment.

4.5 RESPONSE TO THE PROBIOTIC GUM

The different responses to the probiotic gum made it possible to divide the participants into five groups. Three subjects experienced an improvement in the levels of mutans streptococci and lactobacilli in saliva, plaque index, gingival index and saliva flow. Nine subjects had a reduction in both mutans streptococci and lactobacilli levels in saliva. Four of these subjects had lower plaque and gingival indices, one had a reduction in the plaque index and another, the gingival index. In ten subjects there was a reduction in the levels of mutans streptococci but not lactobacilli. Four of these subjects had a reduction in the plaque and gingival indices while one subject had an increase in their saliva flow. Another four participants had an

increase in the levels of mutans streptococci and lactobacilli while in three of these subjects there was also an increase in saliva flow. Finally there was one girl who had a lower plaque index and lactobacilli count but a 15 fold increase in the number of mutans streptococci in saliva which indicates she had developed dental caries during the course of the study because an increase in mutans streptococci is an indication of decay (Jordan, *et al.* 2002).

This varied response to the probiotic gum suggests that the formulation needs to be modified. The formulation of the gum should be reviewed because the placebo gum caused a significant reduction of salivary flow. This is contrary to most published studies on sorbitol gums that increased the salivary flow (Stookey, 2008). Xylitol-sweetened gum is more effective than sorbitol-sweetened gum in reducing caries thus consideration should be given to replacing sorbitol with xylitol in the current formulation (Isotupa, Gunn, Chen, *et al.* 1995). Another suggestion is to increase the number of times the gum is chewed from twice to three times a day and to use two instead of one piece. Further studies are needed to confirm both the results of this study and the success of these proposals.

4.6 MULTIFACTORIAL NATURE OF DENTAL CARIES

This study supports the theory that dental caries is a complex, chronic disease and is strongly influenced by several biological and environmental factors and genetic influences (Werneck, *et al.* 2010). Knowledge of caries-related factors in patients before, during and after active orthodontic treatment helps to determine the caries risk levels and to work out appropriate

individual preventive measures (Sanpei, Endo, Shimooka, 2010). The findings of this study are similar to the findings of Krasse, *et al.* (2006) in that the inter-individual differences were very large and therefore the overall results were not significant. In 26% of the participants chewing the probiotic gum twice daily decreased the salivary microorganisms and plaque and gingival indices. Therefore chewing sugar-free probiotic gum may reduce caries risk in some orthodontic patients. Gum is a supplemental practice, not a substitution for a preventive dental program that includes the use of fluoride, conscientiously applied oral hygiene practices and regular professional visits (Burt, 2006).

4.7 PREVENTION OF WHITE SPOT LESIONS IN ORTHODONTIC PATIENTS

Individuals should be advised to reduce the amount, but more importantly the frequency with which they consume food and drink containing sugar to four times daily (Moynihan & Petersen, 2004). Flossing and brushing teeth after every meal or snack is necessary to remove stagnant areas of plaque and sugars that will be fermented to produce acids and attack the enamel. Chewing xylitol sugar-free gum for a minimum of twice daily would be beneficial. The xylitol in the gum is anticariogenic and the increased salivary flow has various protective effects against demineralisation in orthodontic patients (Isotupa, *et al.* 1995).

This study also supports the idea that the implementation of a caries management system for orthodontic patients is beneficial. Prior to the commencement of treatment, orthodontic practitioners are advised to undertake saliva analyses to identify patients who are at risk of

developing white spot lesions. The analyses should include mutans streptococci and lactobacilli counts in saliva and salivary flow rate and buffering capacity, which should be under control before the placement of orthodontic appliances. Patients should be monitored frequently and a caries management system be adjusted as needed. Professional cleaning and topical fluoride treatments form part of these systems. A xylitol probiotic gum is probably a valuable adjunct in such a program but further research is needed to substantiate claims of its effect.

The rigorous monitoring of diet and oral hygiene should become a part of everyday orthodontic practice. Orthodontic patients should be encouraged to brush and floss their teeth with a fluoride containing toothpaste after every meal or snack. Ongoing coaching and motivation of patients to comply with all requirements of orthodontic treatment is of utmost importance to ensure optimum results. The perceived control in the area of personal health and well-being should be causally related to the patient's own behaviour (Gardiner & Armbruster, 2006).

CHAPTER 5: CONCLUSION

The most striking factor in this study was the inter-individual variation and increased potential to developing white spot lesions. The results of this study suggest that a four week administration of *L. reuteri* (ATCC 55730 and ATCC PTA 5289) probiotic, sugar-free gum may increase the salivary flow rate and reduce the levels of salivary mutans streptococci and lactobacilli and plaque and gingival indices in some orthodontic patients. The present observations merit further studies in order to evaluate the possible positive effects of probiotics in the multifactorial development of white spot lesions and gingival inflammation in orthodontic patients. Further studies are also required to confirm the importance of inter-individual differences in caries susceptibility in orthodontic patients which may be of great benefit to identify those at risk prior to commencement of orthodontic treatment. Nevertheless this study has shown that it is important to devise individualized caries preventative programs for orthodontic patients.

APPENDIX A

List of abbreviations used in text and tables

cfu	Colony forming units
DMFS	Decayed, missing and filled tooth surfaces
g	gram
GI	Gingival index
<i>L.</i>	<i>Lactobacillus</i>
MBA	Mutans Bacitracin Agar
ml	millilitre
PI	Plaque index
ppm	parts per million
RCT	Randomised controlled trial
<i>S.</i>	<i>Streptococcus</i>

APPENDIX B

Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

R14/49 Bronkhorst

CLEARANCE CERTIFICATE

PROTOCOL NUMBER M080612

PROJECT

Effect of Probiotic Chewing Gum on
Streptococcus mutans in Orthodontic
Patients

INVESTIGATORS

Dr A Bronkhorst

DEPARTMENT

Oral Health Sciences

DATE CONSIDERED

08.06.27

DECISION OF THE COMMITTEE*

Approved unconditionally

+

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.06.30

CHAIRPERSON
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof MM Coogan

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX C

Forms for participants

INFORMATION SHEET FOR PARENTS AND LEGAL GUARDIANS

To the Parents and Legal Guardians: *This consent form may contain words that you do not understand. Please ask the study doctor or the study staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.*

Hello

I am Dr Bronkhorst, a dentist specialising in orthodontic at WITS Dental School. I am undertaking a research project on one of the caries producing bacteria, Mutans streptococci, as I believe that the chance of protecting your teeth can be improved by reducing the number of this bacterium in the saliva. This can be achieved by using a mouth rinse and/or a chewing gum in addition to your child's normal oral hygiene routine.

Why are we doing this?

When a patient wears fixed orthodontic appliances there is an increased number of caries producing bacteria in the mouth. Therefore anything that could help protect teeth during orthodontic treatment should be of great benefit to orthodontic patients.

How are we going to do this?

If you agree to let your child participate, you will be given a form to complete requesting details such as their name, age and gender and you will be required to complete a short diet analysis for your child. An oral hygienist will then demonstrate how they should brush and give them toothpaste and a toothbrush. A form will be completed with details of their oral hygiene and oral health status. After that they will be asked to chew on a piece of wax for 10 minutes and then spit all their saliva (spit) in a jar. They will then be given chewing gum which they must chew for 10 minutes twice a day after brushing their teeth. They will do this for 4 weeks after which they will come in for their routine orthodontic assessment appointment. At this appointment your child will again chew on a piece of wax, spit into a jar and their oral hygiene and oral health evaluated. For the next 4 weeks they will be able to "rest" and continue as normal.

After the 4 week "rest" period they will come in for their routine orthodontic assessment appointment. At the same appointment they will again chew on a piece of wax, spit into a jar and their oral hygiene and oral health evaluated. They will then be given a different type of gum which they must chew for 10 minutes twice a day after brushing their teeth. After four weeks, when they once again come in for their routine orthodontic assessment appointment, the same procedures as before will be done. This will be the end of the study.

How and why is your child being invited to participate?

Your child is being invited because he/she wears an orthodontic appliance and as mentioned before when a patient wears fixed orthodontic appliances there is an increased number of caries producing bacteria in the mouth. This study should also make him/her more aware of

the importance of oral hygiene. The treatment may also reduce the chance of developing white spots on his/her teeth.

Is it private?

The personal information on the form will only be used by me and not disclosed to any other parties. Your child's samples will be given numbers and will be processed accordingly. Your child's name will not appear anywhere on the results or in any publications.

Is it safe?

All products that your child will be using are also commercially available and have been tried and tested to be safe and will not affect his/her general health or well being.

May you withdraw your child from the study?

Your child's participation is entirely voluntary. You may withdraw your child from the study at any time. You will not have to explain why you have changed your mind. Your child will not be forced to participate even if you change your mind after saying yes at the beginning. Your child's orthodontic treatment will not be affected by your decision.

When can my child not participate in this study?

If your child has had any systemic antibiotic treatment i.e. taken antibiotic tablets or pills in the last four weeks or at any time during the study they will not be able to take part in or complete the study. If your child has had any topical fluoride treatments (those the oral hygienist or dentist applies) in the last four weeks or at any time during the study they will not be able to take part in or complete the study. If your child has taken a diary probiotic supplement during the last four weeks before the study, he/she will not be able to take part in this study.

Where can I find out more?

If you need any information or have any questions, please contact me, at telephone number 011 488 4896 (W) or e-mail me at adele.bronkhorst@wits.ac.za

If you want your child to participate in the study, please read and sign the attached consent form.

Thank you

Dr. A. Bronkhorst

INFORMATION SHEET FOR PARTICIPANTS

To the Participant: *This consent form may contain words that you do not understand. Please ask the study doctor or the study staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.*

Hello

I am Dr Bronkhorst, a dentist specialising in orthodontic at WITS Dental School. I am undertaking a research project on one of the germs (bacteria) that cause tooth decay (caries). The name of the group of these bacteria which I will be investigating is Mutans streptococci, as I believe that the chance of protecting your teeth can be improved by reducing the number of these germs in the spit (saliva). This can be achieved by using a chewing gum in addition to your normal oral hygiene routine of tooth brushing and flossing.

Why are we doing this?

When a patient wears braces there is an increased number of germs that cause tooth decay in the mouth. Therefore anything that could help protect teeth during orthodontic treatment should be of great benefit to orthodontic patients.

How are we going to do this?

If you agree to participate, you will be given a form to complete requesting the following your name, age and gender. You will be required to complete a 4 day diet analysis. An oral hygienist will demonstrate how you should brush and give you toothpaste and a toothbrush. She will complete a form with details of your oral hygiene and oral health status. After that you will be asked to chew on a piece of wax for 10 minutes and spit into a sterile jar. You will then be given chewing gum which you must chew for 10 minutes twice a day after brushing your teeth. You will do this for 4 weeks after which you will come in for your normal orthodontic assessment appointment. At the same appointment you will again chew on a piece of wax, spit into a jar and your oral hygiene and oral health evaluated. For the next 4 weeks you will be able to “rest”.

After the 4 week “rest” period you will come in for your normal orthodontic assessment appointment. At the same appointment you will again chew on a piece of wax, spit into a jar and your oral hygiene and oral health re-evaluated. You will then be given a different type of gum which you must chew for 10 minutes twice a day after brushing your teeth. After four weeks, when you once again come in for your routine orthodontic assessment appointment, the same procedures as before will be repeated.

How and why are you being invited to participate?

You are being invited because you wear braces and there may be an increased number of germs in the mouth. This study should make you more aware of the importance of caring for your teeth. Chewing special gum may also reduce the chance of developing white spots on your teeth.

Is it private?

The personal information on the form will only be used by me and not disclosed to anyone else. Your samples will be given numbers and your name will not appear anywhere on the results or in any publications.

Is it safe?

All products that you will be using can be bought in the shops and have been tried and tested and will not affect your general health or well being.

May I withdraw from the study?

Your participation is entirely voluntary and you may withdraw from the study at any time. You will not have to explain why you have changed your mind. You will not be forced to take part even if you change your mind after saying yes in the beginning. Your orthodontic treatment will not be affected by your decision.

When can I not participate in this study?

If you have taken antibiotic tablets or pills, a diary probiotic supplement or received topical fluoride treatments in the last four weeks or at any time during the study you will not be able to take part in or complete the study.

Where can I find out more?

If you need any information or have any questions, please contact me, Dr Bronkhorst, at telephone number 011 488 4896 (W) or e-mail me at adele.bronkhorst@wits.ac.za

Thank you

Dr. A. Bronkhorst

INFORMED CONSENT FOR PARENTS/LEGAL GUARDIANS:

(On behalf of minors under 18 years old)

- Dr Bronkhorst has provided me with a copy of the Participant Information Leaflet and Consent regarding clinical study Protocol number M080612 and has fully explained to me the nature, risks, benefits and purpose of the study.
- The study doctor has given me the opportunity to ask any questions concerning both the medicine and the study.
- It has been explained to me that I will be free to withdraw my child from the study at any time, without any disadvantage to future care.
- I have understood everything that has been explained to me and I consent for my child to participate in this clinical study.

PARENT/LEGAL GUARDIAN:

Printed Name

Signature / Mark or Thumbprint

Date and Time

PARTICIPANT ASSENT: * (Seven (7) years old and above)

Printed Name

Signature / Mark or Thumbprint

Date and Time

(* Minors competent to understand must participate as fully as possible in the entire procedure)

STUDY DOCTOR:

Dr Adèle Bronkhorst

Printed Name

Signature

Date and Time

APPENDIX D

Analyses recording forms

DMFS of Participant No _____

F														
M														
D														
	17	16	15	14	13	12	11	21	22	23	24	25	26	27
	47	46	45	44	43	42	41	31	32	33	34	35	36	37
D														
M														
F														

Posterior Teeth: The lingual, mesial, distal and occlusal surfaces will be recorded only once

Anterior Teeth: The labial, lingual, mesial and distal surfaces will be recorded only once

Dental Caries:

The lesion is clinically visible and obvious.

The explorer tip can penetrate into soft yielding material.

Discoloration or loss of translucency typical of undermined or demineralised enamel is apparent.

The explorer tip in a pit or fissure resists removal after moderate to firm pressure on insertion.

D:

- Crown broken down as a result of dental caries
- Caries and a restoration present

M:

- Tooth extracted because of dental caries
- Tooth carious, non-restorable and indicated for extraction

F:

- Permanent and temporary fillings
- A tooth with a defective filling but without evidence of dental caries

Gingival Index of Participant No

4th visit						
3rd visit						
2nd visit						
1st visit						
	16			21	24	
		44	41			36
1st visit						
2nd visit						
3rd visit						
4th visit						

0 = normal gingiva

1 = mild inflammation – slight change in colour, slight oedema but no bleeding on probing

2 = moderate inflammation – redness, oedema and glazing with bleeding on probing

3 = severe inflammation – marked redness and oedema with a tendency to spontaneous bleeding, ulceration of the gingivae may also be present

Plaque Index of Participant No _____

4th Bu						
4th Li						
3rd Bu						
3rd Li						
2nd Bu						
2nd Li						
1st Bu						
1st Li						
	16			21	24	
		44	41			36
1st Bu						
1st Li						
2nd Bu						
2nd Li						
3rd Bu						
3rd Li						
4th Bu						
4th Li						

0 = no plaque

1 = discontinuous band of plaque at the gingival margin

2 = up to 1mm continuous band of plaque at the gingival margin

3 = band of plaque wider than 1mm but less than one-third of the surface

4 = plaque covering one-third or more of the surface, but less than two-thirds of the surface

5 = plaque covering two-thirds or more of the surface

Diet Analysis of

Everything you eat or drink must be entered, including any medication

Approximate quantities of food and drink must be given

Tea or coffee (with or without sugar), cold drinks (regular or diet) must be specified e.g. 1 cup of tea with milk and 3 sugars

	THURSDAY		FRIDAY		SATURDAY		SUNDAY	
	TIME	ITEM	TIME	ITEM	TIME	ITEM	TIME	ITEM
Before Breakfast								
Breakfast								
Morning								
Lunch								
Afternoon								
Dinner								
Bed Time								

REFERENCES

1. Alamoudi N, Farsi N, Faris J, Masoud I, Merdad K, Meisha D (2004) Salivary characteristics of children and its relation to oral microorganism and lip mucosa dryness. The Journal of Clinical Pediatric Dentistry 28:239-248
2. Al Mulla AH, Kharsa SA, Kjellberg H, Birkhed D (2009) Caries risk profiles in orthodontic patients at follow-up using Cariogram. The Angle Orthodontist 79:323-330
3. Al-Omiri MK, Alhaija ESA (2006) Factors affecting patient satisfaction after orthodontic treatment. The Angle Orthodontist 76:422-431
4. Benham AW, Campbell PM, Buschang PH (2009) Effectiveness of pit and fissure sealants in reducing white spot lesions during orthodontic treatment. American Journal of Orthodontics and Dentofacial Orthopaedics 79:338-344
5. Beyth N, Redlich M, Harari D, Friedman M, Steinberg D (2003) Effect of sustained release chlorhexidine varnish on *Streptococcus mutans* and *Actinomyces viscosus* in orthodontic patients. American Journal of Orthodontics and Dentofacial Orthopaedics 123:345-348
6. Boyd RL, Leggott PJ, Quinn RS, Eakle WS, Chambers D (1989) Periodontal implications of orthodontic treatment in adults with reduced or normal periodontal tissues versus those of adolescents. American Journal of Orthodontics and Dentofacial Orthopaedics 96:191-198
7. Burt BA (2006) The use of sorbitol- and xylitol-sweetened chewing gum in caries. Journal of the American Dental Association 137:190-196
8. Çağlar E, Cildir SK, Ergeneli S, Sandalli N, Twetman S (2006) Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium

- Lactobacillus reuteri* ATCC 55730 by straws or tablets. Acta Odontologica Scandinavica 64:314-318
9. Çağlar E, Kavaloglu SC, Kuscu OO, Sandalli N, Holgerson PL, Twetman S (2007) Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutans streptococci and lactobacilli. Clinical Oral Investigations 11:425-429
 10. Çağlar E, Kuscu OO, Cildir SK, Kuvvetli SS, Sandalli N (2008) A probiotic lozenge administered medical device and its effect on salivary mutans streptococci and lactobacilli. International Journal of Paediatric Dentistry 18:35-39
 11. Çağlar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S (2005) Effect of yogurt with Bifidobacterium DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. Acta Odontologica Scandinavica 63:317-20
 12. Chang HS, Walsh LJ, Freer TJ (1997) Enamel demineralization during orthodontic treatment: Aetiology and prevention. Australian Dental Journal 42:322-327
 13. Chang HS, Walsh LJ, Freer TJ (1999) The effect of orthodontic treatment on salivary flow, pH, buffer capacity, and levels of mutans streptococci and lactobacilli. Australian Orthodontic Journal 15:229-234
 14. Cildir SK, Germec D, Sandalli N, Ozdemir FI, Arun T, Twetman S, Çağlar E (2009) Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. European Journal of Orthodontics 31:407-11
 15. Cleaton-Jones P, Richardson BD, Winter GB, Sinwel R, Rantsho JM, Jodaikin A (1984) Dental caries and sucrose intake in five South African pre-school groups. Community Dentistry and Oral Epidemiology 12:381-385

16. Coogan MM, MacKeown JM, Galpin JS, Fatti LP (2008) Microbiological impressions of teeth, saliva and dietary fibre can predict caries activity. *Journal of Dentistry* 36:892-899
17. Corbett JA, Brown LR, Keene HJ, Horton TM (1981) Comparison of *Streptococcus Mutans* concentrations in non-banded and banded orthodontic patients. *Journal of Dental Research* 60:1936-1942
18. Cruickshank R, Duguid JP, Marmion RH, Swain RHA (1975a) pH measurements and buffers, oxidation-reduction potentials, suspension fluids, preparation and glassware. In: *Medical Microbiology the Practice of Medical Microbiology* 12th Ed. London: Churchill Livingstone, p 88
19. Cruickshank R, Duguid JP, Marmion RH, Swain RHA (1975b) Tests for the identification of bacteria. In: *Medical Microbiology the Practice of Medical Microbiology*, 12th Ed. London: Churchill Livingstone p180
20. Curro FA (2004) Gum chewing as an adjunct to use of medications. *Journal of the American Dental Association* 139:6S-8S
21. Dawes C (2008) Salivary flow patterns and the health of hard and soft oral tissues. *Journal of the American Dental Association* 139:18S-24S
22. Delman LA (1955) Effect of gonadectomy on dental caries: review of the literature. *Journal of the American Dental Association* 51:155-158
23. Demling A, Demling C, Schwestka-Polly R, Stiesch M, Heuer W (2010) Short-term influence of lingual orthodontic therapy on microbial parameters and periodontal status: A preliminary study. *The Angle Orthodontist* 80:480-484

24. Derks A, Katsaros C, Frencken JE, Van't Hof MA, Kuijpers-Jagtman AM (2004) Caries-inhibiting effect of preventative measures during orthodontic treatment with fixed appliances. *Caries Research* 38:413-420
25. Derks A, Kuijpers-Jagtman AM, Frencken JE, Van't Hof MA, Katsaros C (2007) Caries preventive measures used in orthodontic practices: An evidence-based decision? *American Journal of Orthodontics and Dentofacial Orthopaedics* 132:165-170
26. Edgar WM (1998) Sugar substitutes, chewing gum and dental caries – a review. *British Dental Journal* 184:29-32
27. Edgar WM, Dawes C, O'Mullane DM (2004) Salivary clearance and its effects on oral health. In: *Saliva and oral health* 3rd Ed. London: British Dental Association
28. el-Ziney MG, Debevere JM (1998) The effect of Reuterin on *Listeria monocytogenes* and *Escherichia Coli* 0157:H7 in milk and cottage cheese. *Journal of Food Protection* 61:1275-1280
29. el-Ziney MG, van den Tempel T, Debevere J, Jakobsen M (1999) Application of reuterin produced by *Lactobacillus reuteri* 12002 for meat decontamination and preservation. *Journal of Food Protection* 62:257-61
30. Fejerskov O, Thylstrup A (1994) Different concepts of dental caries and their implications. In: *Cariology* 4th Ed. Copenhagen: Munksgaard, pp209-217
31. Foley T, Aggarwal M, Hatibovic-Kofman S (2002) A comparison of the *in vitro* enamel demineralization potential of three orthodontic cements. *American Journal of Orthodontics and Dentofacial Orthopaedics* 121:526-530
32. Forsberg CM, Brattström V, Malmberg E, Nord CE (1991) Ligature wires and elastomeric rings: two methods of ligation, and their association with microbial

- colonization of *Streptococcus mutans* and *lactobacilli*. *European Journal of Orthodontics*. 13:416-420
33. Gänzle MG, Hölzel A, Walter J, Jung G, Hammes WP (2000) Characterisation of reutericyclin produced by *Lactobacillus reuteri* LTH2584. *Applied and Environmental Microbiology* 66:4325-33
 34. Gardiner DM, Armbruster PC (2006) Psychosocial behavioral patterns for adolescents. *Dental Clinics of North America* 50:17-32
 35. Geiger AM, Gorelick L, Gwinnett AJ, Benson BJ (1992) Reducing white spot lesions in orthodontic populations with fluoride rinsing. *American Journal of Orthodontics and Dentofacial Orthopaedics* 101:403-407
 36. Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG (1988) The effect of a fluoride program on white spot formation during orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopaedics* 93:29-37
 37. Gold OG, Jordan HV, Van Houte J (1973) A selective medium for *Streptococcus Mutans*. *Archives of Oral Biology* 18:1357-1364
 38. Gorelick L, Geiger AM, Gwinnett AJ (1982) Incidence of white spot formation after bonding and banding. *American Journal of Orthodontics* 81:93-98
 39. Gray A, Ferguson MM (1996) The use of low-tack chewing gum for individuals wearing orthodontic appliances. *Australian Dental Journal* 41:373-376
 40. Haukioja A, Loimaranta V, Tenovu J (2008) Probiotic bacteria affect the composition of salivary pellicle and streptococcal adhesion *in vitro*. *Oral Microbiology and Immunology* 23:336-343
 41. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) In: *Bergeys manual for determinative bacteriology*. 9th Ed. Baltimore: Williams & Wilkens

42. Huang GJ (2004) Occlusal adjustment for treating and preventing temporomandibular disorders. *American Journal of Orthodontics and Dentofacial Orthopaedics* 126:138-139
43. Imfeld T (1999) Chewing gum_facts and fiction: A review of gum-chewing and oral health. *Critical Reviews in Oral Biology and Medicine*. 10:405-419
44. Isotupa KP, Gunn S, Chen C-Y, Lopatin D, Mäkinen (1995) Effect of polyol gums on dental plaque in orthodontic patients. *American Journal of Orthodontics and Dentofacial Orthopaedics* 107:497-504
45. Jordan C, LeBlanc DJ (2002) Influences of orthodontic appliances on oral populations of mutans streptococci. *Oral Microbiology and Immunology* 17:65-71
46. Jones , Kedwar (1989) In: *Design and Analysis of Cross-over Trials* London, Chapman & Hall, pp16-85
47. Kidd EAM (2005) Diet and Caries. In: *Essentials of Dental Caries* 3rd Ed. New York: Oxford University Press, pp94-98
48. Kidd EAM, Joyston-Bechal S (1987) Diagnosis and its Relevance to Management. In: *Essentials of Dental Caries : The disease and its management* Bristol: Wright, pp41-57
49. Kleemola-Kujala E, Räsänen L (1982) Relationship of oral hygiene and sugar consumption to risk of caries in children. *Community Dentistry and Oral Epidemiology* 10:224–33
50. König KG, Navia JM (1995) Nutritional role of sugars in oral health. *The American Journal of Clinical Nutrition*. 62:275S-282S
51. Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G (2006) Decreased gum bleeding and reduced gingivitis by the probiotic *Lactobacillus reuteri*. *Swedish Dental Journal* 30(2):55-60

52. Kupietzky A, Majumdar AK, Shey Z, Binder R, Matheson PB (2005) Colony forming unit levels of salivary lactobacilli and Streptococcus mutans in orthodontic patients. *Journal of Clinical Pediatric Dentistry* 30:51-53
53. Langenhoven ML, Kruger M, Gouws E, Faber M (1991) In: *Research Institute for Nutritional Diseases Food Composition Tables* 3rd Ed, Cape Town: South African Medical Research Council pp1-248
54. Law V, Seow WK, Townsend G (2007) Factors influencing oral colonization of mutans streptococci in young children. *Australian Dental Journal* 52:93-100
55. Lorenz K, Bruhn G, Brex M, Toutenburg H, Heumann C, Netuschil L, Hoffmann T (2007) Full mouth versus partial-mouth recording in experimental gingivitis studies. Poster Presentation IADR/AADR/CADR 85th General Session and Exhibition
56. Mäkinen KK, Chen CY, Mäkinen PL, Bennett CA, Isokangas PJ, Isotupa KP, Pape HR Jr. (1996) Properties of whole saliva and dental plaque in relation to 40 month consumption of chewing gums containing xylitol, sorbitol or sucrose. *Caries Research* 30:180-188
57. MatosGomes N, Katsurayama M, Makimoto FH, Santana LLO, Paredes-Garcia E, Becker MAdA, Dos-Santos MC (2010) Psychological stress and its influence on salivary flow rate, total protein concentration and IgA, IgG and IgM titers. *Neuroimmunomodulation* 17:396-404
58. Matsukubo T, Takazoe I (2006) Sucrose substitutes and their role in caries prevention. *International Dental Journal* 56:119-130
59. Mitchell L (1992) Decalcification during orthodontic treatment with fixed appliances – an overview. *British Journal of Orthodontics* 19:199-205

60. Mizrahi E (1982) Enamel demineralization following orthodontic treatment. *American Journal of Orthodontics* 82:62-67
61. Mobley CC (2003) Nutrition and dental caries. *Dental Clinics of North America* 47:319-336
62. Montalto M, Vastola M, Marigo L, Covino M, Graziosetto R, Curigliano V, Santoro L, Cuoco L, Manna R, Gasbarrini G (2004) Probiotic treatment increases salivary counts of lactobacilli: a double-blind, randomized controlled study. *Digestion*. 69:53-56
63. Morrow D, Woode DP, Speechley M (1992) Clinical effect of subgingival chlorhexidine irrigation on gingivitis in adolescent orthodontic patients. *American Journal of Orthodontics and Dentofacial Orthopaedics* 101:408-413
64. Moynihan PJ (2002) Dietary advice in dental practice. *British Dental Journal* 193:563-568
65. Moynihan PJ, Petersen PE (2004) Diet, nutrition and the prevention of dental diseases. *Public Health Nutrition* 7(1A):201-226
66. Newbrun E (1989) Current concepts of caries etiology. In: *Cariology* 3rd Ed. Chicago: Quintessence, pp29-61
67. Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K, Darmawan S, Hamada T, Hara K, Matsumoto A, Takemoto T, Aimi R (2004) *Lactobacillus reuteri* in bovine milk fermented decreases the oral carriage of mutans streptococci. *International Journal of Food Microbiology* 95:219-223
68. Øgaard B (1989) Prevalence of white spot lesions in 19-year-olds: A study on untreated and orthodontically treated persons 5 years after treatment. *American Journal of Orthodontics and Dentofacial Orthopaedics* 96:423-427

69. Øgaard B, Rølla G, Arends J, ten Cate JM (1988) Orthodontic appliances and enamel demineralization. Part 2. Prevention and treatment of lesions. *American Journal of Orthodontics and Dentofacial Orthopaedics* 94:123-128
70. Oh TJ, Eber R, Wang HL (2002) Periodontal diseases in the child and adolescent. *Journal of Clinical Periodontology* 29:400-410
71. Park JH, Tanabe Y, Tinanoff N, Turng BF, Lili H, Minah GE (2006) Evaluation of microbiological screening systems using dental plaque specimens from young children aged 6-36 months. *Caries Research* 40:277-280
72. Pender N (1986) Aspects of oral health in orthodontic patients. *British Journal of Orthodontics* 13:95-103
73. Percival RS, Chalacombe SJ, Marsh PD (1994) Flow rates of resting whole and stimulated parotid saliva in relation to age and gender. *Journal of Dental Research* 73:1416-1420
74. Russell C, Ahmed FI (1978) Interrelationships between lactobacilli and streptococci in plaque formation on a tooth in an artificial mouth. *Journal of Applied Bacteriology* 45:373-382
75. Sakamaki ST, Bahn AN (1968) Effect of orthodontic banding on localised oral lactobacilli. *Journal of Dental Research* 47:275-279
76. Sanpei S, Endo T, Shimooka S (2010) Caries risk factors in children under treatment with sectional brackets. *The Angle Orthodontist* 80:509-514
77. Sari E, Birinci I (2007) Microbiological evaluation of 0.2% chlorhexidine gluconate mouth rinse in orthodontic patients. *The Angle Orthodontist* 77:881-884

78. Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM (2005) Probiotic and other functional microbes: from markets to mechanisms. *Current Opinion in Biotechnology* 16:204-211
79. Scheie AA, Arneberg P, Krogstad O (1984) Effect of orthodontic treatment on prevalence of *Streptococcus mutans* in plaque and saliva. *Scandinavian Journal of Dental Research* 92:211-217
80. Schwaninger B, Vickers-Schwaninger N (1979) Developing an effective oral hygiene program for the orthodontic patient: review, rationale, and recommendations. *American Journal of Orthodontics* 75:447-52
81. Signoretto C, Bianchi F, Burlacchini G, Sivieri F, Spratt D, Canepari P (2010) Drinking habits are associated with changes in the dental plaque microbial community. *Journal of Clinical Microbiology* 48:347-356
82. Stecksén-Blicks C, Arvidsson S, Holm A-K (1985) Dental health, dental care and dietary habits in children in different parts of Sweden. *Acta Odontologica Scandinavica* 43:59–67
83. Stookey GK (2008) The effect of saliva on dental caries. *Journal of the American Dental Association* 139;11S-17S
84. Streckfus CF, Baur U, Brown LJ, Bacal C, Metter J, Nick T (1998) Effects of estrogen status and aging on salivary flow rates in healthy Caucasian women. *Gerontology* 44:32-39
85. Sudjalim TR, Woods MG, Manton DJ (2006) Prevention of white spot lesions in orthodontic practice: a contemporary review. *Australian Dental Journal* 51:284-289
86. Svanberg MS (1980) Mutans in plaque after mouth-rinsing with buffers of varying pH value. *Scandinavian Journal of Dental Research* 88:76-78

87. Talarico TL, Casas IA, Chung TC, Dobrogosz WJ (1988) Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus Reuteri*. *Antimicrobial Agents Chemotherapy* 32:1854-1858
88. Touger-Decker R, Van Loveren C (2003) Sugars and dental caries. *American Journal of Clinical Nutrition* 78:881S-892S
89. Turesky S, Gilmore ND, Glickman I (1970) Reduced plaque formation by the chloromethyl analogue of vitamin C. *Journal of Periodontology* 41:41-43
90. Twetman S, Stecksén-Blicks C (2007) Probiotics and oral health effects in children. *International Journal of Paediatric Dentistry* 18:3-10
91. Ulukapi H, Koray F, Efes B (1997) Monitoring the caries risk of orthodontic patients. *Quintessence International* 28:27-29
92. Van Houte J, Gibbons RJ, Pulkkinen AJ (1972) Ecology of human oral lactobacilli. *Infection and Immunity* 6:723-729
93. Van Loveren C (2004) Sugar alcohols: What is the evidence for caries preventive and caries-therapeutic effects? *Caries Research* 38:286-293
94. Werneck R, Mira M, Trevilatto P (2010) A critical review: an overview of genetic influence on dental caries. *Oral Diseases* 2010 May 30. [Epub ahead of print]
95. Whiley RA, Beighton D (1998) Current classification of oral streptococci. *Oral Microbiology and Immunology* 13:195-216
96. Wilkins EM (1999) Indices and Scoring Methods. In: *Clinical Practice of the Dental Hygienist*. 8th ed. Philadelphia: Lippincott, pp293-313
97. Yip HH, Wong RW, Hägg U (2009) Complications of orthodontic treatment: are soft drinks a risk factor? *World Journal of Orthodontics* 10:33-40

98. Zachrisson BU, Zachrisson S (1971) Caries incidence and orthodontic treatment with fixed appliances. *Scandinavian Journal of Dental Research* 79:183–192
99. Zachrisson BU (1975) Fluoride application procedures in orthodontic practice, current concepts. *The Angle Orthodontist* 45:72-81
100. Zachrisson BU (1976) Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment. *American Journal of Orthodontics* 69:285-300
101. Zachrisson BU (1977) A posttreatment evaluation of direct bonding in orthodontics. *American Journal of Orthodontics* 71:173-189