The effects of Chlorhexidine containing toothpastes and Tea Tree Oil containing mouthwashes on plaque and gingival inflammation

A thesis submitted in partial fulfilment for
the Degree of Masters of Dental Surgery (Periodontics)

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This is dedicated to my family,
especially my parents,
sister,
brothers and
nephew

Signed Statement

This research report is submitted in partial fulfilment of the requirements of the Degree of Master of Dental Surgery (Periodontics) in the University of Adelaide.

This study contains no material that has been accepted for the award of any other degree or diploma in any university or any other tertiary institution. To the best of my knowledge and belief, it contains no other material previously published or written by another person except when due reference is made in the text of the report.

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Summary

This study tested the plaque inhibitory effects of a newly formulated chlorhexidine toothpaste; and the plaque inhibitory and anti-gingivitis effects of a mouthwash containing tea tree oil.

(1) Chlorhexidine toothpaste

The aim of this study was to evaluate the effect of a newly formulated chlorhexidine containing toothpaste on plaque formation and the amount of discolouration of teeth using the four day plaque growth model as described by Addy et al (1983). The efficacy of chlorhexidine mouthwash in preventing plaque accumulation is well documented. Considering that toothbrushing combined with the use of toothpaste is the most commonly used form of oral hygiene, it seems logical to develop a toothpaste containing a proven antiseptic. Toothpastes containing chlorhexidine have had limited plaque inhibitory activity and the results of this study concur with those of previous studies (Johansen et al. 1975; Dolles et al. 1979). However, these results are in contrast to another study which reported a reduction in gingivitis when compared to a placebo (Sanz et al. 1994).

One chlorhexidine containing toothpaste was tested in a blind crossover randomised 4 day plaque growth model (Addy et al. 1983) with a washout period of at least 16 days between preparations. Plaque was scored using the Quigley and Hein Plaque Index (1962). Thirty healthy non-smoker subjects completed the trial. The ranking from the lowest to highest plaque index score was:

- 0.12% chlorhexidine mouthwash (the positive control),

- chlorhexidine toothpaste,
- Colgate Total® and
- the chlorhexidine toothpaste base with no active agent.

Stain was scored using the Discolouration Index System (DIS) by Lang and Raber (1981). The ranking from the highest to lowest stain index score was:

- 0.12% chlorhexidine mouthwash (positive control),
- chlorhexidine toothpaste,
- the chlorhexidine toothpaste base with no active agent and
- Colgate Total®.

Statistical analysis by t-tests showed that there was no significant difference between plaque index scores of the chlorhexidine containing toothpaste and Colgate Total®. All other comparisons were significantly different.

The chlorhexidine containing toothpaste did not exhibit the pronounced plaque inhibitory effect that would be expected of a chlorhexidine containing agent. It is likely that the chlorhexidine in the toothpaste was either inactivated by, chemically bound to, or in competition with other ingredients in the toothpaste.

(2) Tea tree oil mouthwash (TTO)

The aim of this study was to evaluate the effect of a TTO mouthwash on plaque formation, and on the amount of discolouration of oral structures, again using the four day plaque growth model and the effect of one TTO mouthwash on gingival health in a 6 week home use study. Preparations tested in the 4 day plaque growth study were the

TTO containing mouthwash, Listerine®, 0.12% chlorhexidine mouthwash and a mouthwash base. In the 6 week trial, the TTO mouthwash was tested against a mouthwash base.

TTO is a naturally occurring antibacterial which has been used as a disinfectant for many decades. TTO mouthwash was tested in a blind crossover randomised 4 day plaque growth model with a washout period of at least 16 days. Twenty five healthy non-smoking subjects completed the trial. The same plaque and stain indices were used here as with the trial before. The ranking from the lowest to the highest plaque scores was:

- TTO mouthwash,
- Listerine® ,
- 0.12% chlorhexidine mouthwash and
- placebo.

The ranking of the stain scores from highest to lowest was:

- TTO mouthwash,
- 0.12% chlorhexidine mouthwash ,
- Listerine® and
- placebo.

There was no significant difference between the plaque inhibitory effects of TTO mouthwash and Listerine®.

The longer term effects on oral health of TTO mouthwash over 6 weeks were compared to a placebo, and assessed using the plaque, papillary bleeding and gingival indices.

Forty nine healthy non-smokers completed this trial. The TTO plaque score decreased and stain score increased significantly over 6 weeks when compared with the placebo. The TTO was not significantly different from the placebo with regard to the gingival and papillary bleeding index scores. As with the TTO mouthwash in the 4 day plaque growth study, other plaque inhibitory agents had been added to the TTO test mouthwash. The suppliers were responsible for the composition of the TTO mouthwash and it was revealed at the completion of the trial that other antiseptic agents had been included with the TTO. The supplier had added triclosan and cetylpyridinium chloride to TTO mouthwash which was tested in both the randomised 4 day plaque growth and 6 week long term studies. In addition, the chlorhexidine mouthwash positive control had been supplied in an inactive form. This rendered the trial involving TTO mouthwash of little value in regard to scientific evidence about the plaque inhibitory effects of TTO. Further research is required to test the TTO agent on plaque and oral health independently from other plaque inhibitory agents.

Collecting information about plaque levels, oral staining and gingival health is a time consuming process in large scale clinical trials. Reducing the number of teeth scored, or the tooth surface scored (or both) would make trials easier to carry out, provided that teeth/surfaces data sets were reflective of the whole mouth score. Therefore, it was decided to compare the analyses of data using different data sets such as that of 28 and 20 teeth, and for buccal and lingual surfaces. Different data sets were compared in order to establish the minimum number of teeth / tooth surfaces that can be used in future studies that still are representative of whole mouth scores.

Different sets of data were analysed using the mean total score (a maximum of 56 readings - buccal and lingual surfaces of 28 teeth divided by 56); 28 teeth analysis (which was the average of 12 scores - buccal and lingual surfaces of anterior and posterior teeth in the maxilla and mandible), and 20 teeth analysis (which was the average of 12 scores - buccal and lingual surfaces of incisors and canine/premolars in the maxilla and mandible)

The ranking of preparations in the 4 day plaque trial were listed in the previous pages. This ranking in relation to the individual indices for the total mean scores were reflected in the following data sets:

- plaque index - 28 and 20 teeth mean score, mandibular teeth score in 28 teeth analysis, lingual surfaces in 28 and 20 teeth analysis, 20 teeth maxillary score;
- stain index - 28 and 20 teeth mean score, 28 and 20 teeth mean score, mandibular teeth score in 28 teeth analysis;
- gingival index - 28 and 20 teeth mean score, and mandibular teeth score in 28 teeth analysis.
- bleeding index - no other data sets showed the same results in terms of ranking of preparations with the total mean score.

These data sets may provide the same results (in terms of ranking) for each index in future studies.

A new plaque index to better score plaque coverage and sparseness was developed, but it has not been tested.

In conclusion,

- chlorhexidine toothpaste was significantly different to chlorhexidine mouthwash in its plaque inhibitory activity in the 4 day plaque growth study (ie. the chlorhexidine toothpaste was less effective than the chlorhexidine mouthwash);
- TTO mouthwash was significantly different from the placebo in the 6 week long term use study.

TTO mouthwash could not be analysed against chlorhexidine mouthwash in the 4 day plaque growth study because the chlorhexidine mouthwash had been supplied in an inactivated form.

Future recommendations are:

- to test the effectiveness of the plaque index developed from this study;
- to further develop chlorhexidine toothpaste formulations to liberate the true plaque inhibitory potential of chlorhexidine;
- to conduct a study to test the true plaque inhibitory activity of TTO; and
- to test the contents of industry-supplied mouthwashes and other preparations prior to issue.

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Quote

" I never take a walk with three persons,
without finding that one of them has something to teach me ..."

" To know what you know and know what you don't know
is the characteristic of one who knows ..."

CONFUCIUS

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List of Abbreviations

TTO	tea tree oil
ch	chlorhexidine
t p	toothpaste
m w	mouthwash
sig	significant

Chapter 1

INTRODUCTION

Plaque control is essential for the maintenance of optimal oral health, being important in the prevention of gingivitis and caries. Mechanical plaque removal, such as tootbrushing, is the most widely practised form of oral hygiene but it rarely results in complete plaque removal because most people are not sufficiently motivated or dextrous. In addition, situations where people are unable to remove plaque by conventional means dictate that alternative methods of plaque control are required.

Therefore, research into safe and effective chemotherapeutic agents as adjuncts to mechanical plaque removal has become popular (De Paola et al. 1989; Overholser et al. 1990). These agents exert plaque inhibitory effects either by removing the plaque already formed, altering the already formed plaque or by preventing the formation of new plaque (Addy 1997).

The characteristics of the ideal plaque inhibitory agent include:

- the ability to reduce plaque formation without permanently altering the microbial flora (ie. induce the development of resistant bacteria);
- minimal side effects (both local and systemic);
- high substantivity with plaque inhibitory action over a prolonged period;
- no loss of activity when incorporated into a dentrifice;
- acceptable taste;
- local action;
- absence of toxic breakdown products;

- non-toxic metabolism and ready elimination by the body.

Chemotherapeutic agents that have been investigated include enzymes, bisbiguanides, quarternary ammonium compounds, essential oils, natural products (sanguinarine), fluorides, metal salts, oxygenating agents, detergents, amine alcohol and antibiotics (Addy 1997). Most of these products have limited use due to their side-effects at therapeutic doses. Antibiotics such as tetracycline have also been tested for their plaque inhibitory effects but the high systemic doses required and the development of bacterial resistance preclude their long term use.

The two products tested in this study were a chlorhexidine containing toothpaste and a mouthwash containing tea tree oil (TTO).

Chlorhexidine is a bisbiguanides, and in mouthwash form is considered to be the 'gold standard' of plaque inhibitory agents (Addy 1997). However, chlorhexidine containing toothpastes have shown only moderate plaque inhibitory activity to date (Johansen et al. 1975; Sanz et al. 1994). The antimicrobial action of TTO has been reported in a few studies (Walsh and Longstaff 1987; Carson and Riley 1993; Carson and Riley 1994; Shapiro et al. 1994; Carson and Riley 1995; Raman et al. 1995; Rogers and Gully 1999). However, little scientific research has been conducted into the clinical efficacy of this product other than microbiological studies against oral bacteria (Shapiro et al. 1994; Rogers and Gully 1999).

This was a controlled study in three parts:

- Part 1: 30 subjects testing the plaque inhibitory activity of chlorhexidine toothpaste;

- Part 2: 25 subjects testing the plaque inhibitory activity of tea tree oil mouthwash; and
- Part 3: 49 subjects testing the anti-gingivitis activity of tea tree oil mouthwash

Parts 1 and 2 of this study used the 4 day plaque growth design (Addy et al. 1983), and to utilise Quigley and Hein (1962) plaque index and Lang and Raber (1981) discolouration index to score plaque and stain accumulation respectively. Part 3 aimed to follow long term home use utilising the Löe (1967) Gingival Index and Mühlemann (1977) Papillary Bleeding Index to measure the effects of TTO mouthwash on gingival health, in addition to measuring plaque and stain changes.

The main aims of this study were to determine:

- the plaque inhibitory effects of a chlorhexidine containing toothpaste when used as a slurry twice a day in a four day plaque growth model;
- the plaque inhibitory effects of TTO containing mouthwash, when used twice a day in a four day plaque growth model;
- the effects of TTO containing mouthwash on chronic gingivitis in a 6 week home use model.

The secondary aims of this study were to determine:

- the amount of stain associated with the use of the chlorhexidine containing toothpaste over 4 days;
- the amount of stain associated with the use of the TTO containing mouthwash over 4 days and 6 weeks;

- the subjective taste acceptability associated with the use of the chlorhexidine containing toothpaste over 4 days;
- the subjective taste acceptability associated with the use of the TTO containing mouthwash over 4 days and 6 weeks;
- the surfaces and number of teeth which best correlate clinical and statistical significance.

These issues will be discussed in detail in separate papers and are not the primary scope of this thesis.

The null hypotheses (H_o) for this study were:

H_o 1: There is no difference between chlorhexidine toothpaste and 0.12% chlorhexidine mouthwash in their plaque inhibitory action.

H_o 2: There is no difference between tea tree oil mouthwash and 0.12% chlorhexidine mouthwash in their plaque inhibitory action.

H_o 3: There is no difference between tea tree oil mouthwash and placebo mouthwash in their effect on gingival health.

Chapter 2

LITERATURE REVIEW

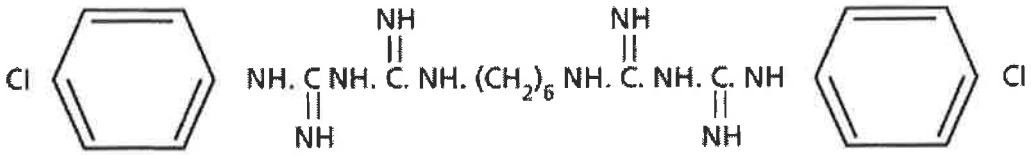
The main classes of plaque inhibitory agents are the cationic compounds (bisguanides, quarternary ammonium compounds, pyrimidine derivatives, bispyridine derivatives); phenolics (essential oils and triclosan); herbal extracts (sanguinarine), heavy metal salts (silver, mercury, tin, zinc and copper); enzymes (mutanase, dextranase); anionic surfactants and oxygenating agents (peroxides , perborate) (Hennessey 1977; Newbrun 1989; Heasman and Seymour 1994; Fine 1995). Chlorhexidine is a bisguanide, and TTO is an essential oil.

2.1 CHLORHEXIDINE

Chlorhexidine exists in three salt forms: digluconate, acetate and hydrochloride (Gjermo et al. 1974; Addy and Hunter 1987; Ross et al. 1989; Schaeken et al. 1994; Addy 1997). It has been used as a topical antiseptic in the medical field since the 1950s (Rushton 1977). Uses include pre-surgical skin preparation, treatment of burns and prior to obstetrical/gynaecological procedures. There are few reports of adverse reactions or sensitisation to this chemical. Chlorhexidine's plaque inhibitory properties have been researched since around the middle of the century (Schroeder 1969). The efficacy of chlorhexidine mouthwash efficacy has been evaluated extensively in the literature. Considering that toothbrushing (and the use of toothpaste) is the most commonly used form of oral hygiene, it seems logical to develop a toothpaste which incorporates a proven antiseptic, such as chlorhexidine.

Chemistry

The chlorhexidine molecule is a symmetrical cationic molecule, consisting of two 4-chlorophenyl rings and two biguanine groups connected by a central hexamethylene chain (Bain 1980). Its most stable salt (the digluconate) is a strong base (Case 1977), and is highly soluble and dicationic above pH 3.5. In addition to its hydrophilic nature, it is also lipophilic (Bonesvoll 1977).



1,6–di (4–chlorophenyldiguanido) hexane

Figure 2.1 Chlorhexidine molecule.

Clinical efficacy

As a plaque inhibitory agent, chlorhexidine mouthwash is superior to fluoride (Jenkins et al. 1993; Joyston-Bechal and Hernaman 1993), essential oils (Overholser et al. 1990), triclosan (Schaeken et al. 1994), and phenolic and sanguinarine products (Grossman et al. 1989). The plaque inhibitory properties of chlorhexidine result in reduced supragingival plaque accumulation, adult gingivitis and (Bain 1980) possibly the incidence of caries (Johansen et al. 1975).

Chlorhexidine mouthrinse (0.2% twice daily) is considered to be the 'gold standard' of chemical supragingival plaque control agents (Gjermo et al. 1974; Addy and Hunter 1987; Ross et al. 1989; Schaeken et al. 1994). Plaque inhibition by chlorhexidine is related to its frequency of application (Mendieta et al. 1994).

Toxicity

Chlorhexidine readily attaches to mucous membranes, but is not readily absorbed from the mucosa of the oral cavity and the gastro-intestinal tract (GIT) (Bain 1980). Chlorhexidine does not cause adverse reactions when accidentally given intravenously; is not readily absorbed through skin; its absorption from the gastrointestinal tract was shown to be low. Chlorhexidine solutions of up to 0.2% concentration are well tolerated by humans. Para-chloroaniline, a known carcinogen, is a breakdown product when of chlorhexidine is stored for prolonged periods at high temperature. Fortunately, absorption studies and faecal analyses have revealed that the chlorhexidine does not degrade to this simple molecule of aromatic systems. Chlorhexidine is assumed to be excreted in equal amounts in urine and bile (Bain 1980). Animal studies using whole body autoradiography following oral administration show very little trace of the substance in tissues. These findings confirm the fact that chlorhexidine remains intact along the GIT. Inadvertent ingestion results in the excretion via faeces and the kidneys in its intact form (Bain 1980).

About 9-18mg of chlorhexidine reaches the gastro-intestinal tract when subjects rinsed twice daily with 10ml of 0.2% concentration of chlorhexidine (Bonesvoll et al. 1974). The low pH of gastric juices would un-ionise the acidic groups of albumin and other proteins and therefore, there is insignificant binding of chlorhexidine to protein in the GIT. After oral use of chlorhexidine, systemic absorption is minimal and does not result in detectable blood levels (Case 1977; Rushton 1977). Studies involving the use of labelled chlorhexidine molecules also show that metabolic cleavage of the molecule does not occur (Rushton 1977). The long term use of chlorhexidine has been deemed safe from a chemical point of view.

Retention and Binding

The prolonged retention of chlorhexidine in the oral cavity is referred to as its substantivity. The high substantivity of chlorhexidine allows a prolonged plaque inhibitory action. Retention of chlorhexidine in the oral cavity is the most important factor in its plaque inhibitory action (Rölla and Melsen 1975). Retention of chlorhexidine in the oral cavity is related to its adsorption onto the oral surfaces (Jenkins et al. 1988). Approximately one third of the chlorhexidine is retained in the oral cavity, binding to the plaque on hard dental structures, and to acidic molecules on pellicle, plaque, and mucous membranes (Bain 1980). The cationic properties of chlorhexidine facilitates this binding (Fardal and Turnbull 1986).

The degree of retention of chlorhexidine in the oral cavity is pH dependent (Hjeljord et al. 1973; Gjermo et al. 1974; Rölla and Melsen 1975; Bonesvoll 1977). When the pH was lowered to pH 1.5-3, a marked decrease in clinical effect was seen (Gjermo et al. 1974). With low pH, numerous hydrogen ions probably reduced the number of negatively charged binding sites (such as the carboxyl, sulphate and phosphate groups) on oral structures. The effects of acidic conditions on chlorhexidine suggests that its retention is dependent on its binding to proteins. Perhaps it is the carboxyl groups on mucin layers which bind chlorhexidine. The carboxyl groups are undissociated at pH 3, whereas the sulphate and phosphate groups remain charged. Salivary sulphated groups also provide binding sites to facilitate the retention of chlorhexidine in the oral cavity. About a third of the chlorhexidine retained in the oral cavity is bound to phosphate groups, and much of this is to mucous membrane surfaces (Fardal and Turnbull 1986). Salivary chlorhexidine levels displayed a logarithimic fall during the first 4-8 hours following administration (Bonesvoll 1977). They were still detectable after 24 hours

(Bain 1980). Traces of chlorhexidine have been detected in the oral cavity up to a week after a single rinse with chlorhexidine (Emilson et al. 1973). Residual salivary antibacterial activity remained for up to 5 hours (Roberts and Addy 1981). The binding to the carboxyl groups, present on sialic acid in salivary glycoproteins appears to be a major retention factor of chlorhexidine in the oral cavity (Rölla and Melsen 1975). Sulphate binding sites are present on sulphated glycoproteins in mucous salivary secretions. Phosphate groups are present on bacterial surfaces, and on a phosphoprotein produced by the parotid gland.

In vitro, the binding of chlorhexidine in saliva has been shown to involve albumin (Hjeljord et al. 1973). This binding is also concentration dependent and occurs to protein both in solution and precipitated. Extrapolation from these *in vitro* experiments could suggest a possible explanation as to the retention of chlorhexidine to the glycoprotein layer on tooth structure in the mouth, despite the rapid turnover of saliva. At pH 3.0, the acidic groups of albumin would be un-ionised and unavailable for salt binding. When the pH is increased from pH 8 to 9, a dramatic increase in binding was observed. This may be explained by the loss of positive charge from the amino groups, which resulted in a higher negative charge on the protein. High pH also increases the lipid solubility of chlorhexidine molecules also influencing the formation of chlorhexidine-protein complexes. The high pH may also alter the configuration of protein, and increase the number of binding sites. The fact that chlorhexidine is a strong base may explain why protein-chlorhexidine complexes are highly insoluble. Coincidently, the concentration at which albumin is precipitated by chlorhexidine, is the same concentration at which the latter has its clinical effectiveness. The binding of

chlorhexidine to proteins in solution and to precipitated proteins are both reversible, although there is a stronger bond to the latter.

Chlorhexidine is reported to saturate hydroxyapatite at an uptake of about 18 micro mole per gram of apatite (Emilson et al. 1973). Multiple layers were formed by chlorhexidine on apatite when the concentration of the mouthwash was varied. A stable monolayer was formed when 0.005-0.01% chlorhexidine was applied topically, indicating that the uptake of chlorhexidine was related to its administered concentration.

Electrostatic bonds contribute to the binding of chlorhexidine in the oral cavity (Bonesvoll 1977). Chlorhexidine has been shown to bind to bacteria, extracellular polysaccharide, and salivary proteins *in vitro*. Anionic agglutinating factors have also been shown to be present in plaque. Chlorhexidine binds to hydroxyapatite, and to acrylic dentures. The retention of chlorhexidine in the oral cavity is approximately directly proportional to the administered concentration. It was observed to be retained quickly in the first 15 seconds of rinsing, and then its retention slowed down. From these observations, multiple rinses of short duration would probably lead to increased retention of chlorhexidine in the oral cavity, and its subsequent plaque inhibitory action. A rinse of 0.05% was still shown to have good plaque inhibitory effects in this study (Bonesvoll 1977). The binding of chlorhexidine is influenced by hydrogen bonds in addition to its flexible molecular structure which enables it to reconfigure and attach many different binding sites. A common dentrifice detergent, sodium dodecyl lauryl sulphate at 25mM markedly reduces the retention and plaque inhibitory effects of chlorhexidine mouthwash. This detergent probably forms an insoluble complex with chlorhexidine, which inactivates the chlorhexidine. Glycoproteins are usually bound to

the mucosa and aid in the retention of chlorhexidine. However, detergents effectively solubilise these glycoproteins and cause the glycoproteins to dissociate from the mucosa. Therefore chlorhexidine bound to glycoproteins can be inadvertently expectorated resulting in decreased retention of chlorhexidine. The presence of teeth did not appear to influence the amount of retention of chlorhexidine (Bonesvoll and Olsen 1974). This may be due either to the insensitivity of the measurement techniques or individual variation in uptake of chlorhexidine.

Glucosyltransferases (GTF) are involved in the formation of plaque. Both the bound and extracellular GTF have been found in saliva and in pellicle. One method by which to reduce plaque is to inhibit glucan synthesis by non-cell bound GTF. Chlorhexidine was shown to inhibit glucan formation by GTF from saliva. This inhibition effectively reduces plaque formation. GTF which has been exposed to chlorhexidine may still bind to hydroxyapatite, but is inactivated. *In vivo* studies have shown some level of reduced activity of GTF in saliva (Scheie and Kjeilen 1987).

Summary of factors involved in the retention of chlorhexidine

- chlorhexidine binds to oral surfaces
- chlorhexidine binds to salivary glycoproteins and plaque
- chlorhexidine binds to bacteria
- acidic pH decreases retention of chlorhexidine
- detergent in toothpastes interacts with chlorhexidine to form an insoluble salt

Mechanism of action of chlorhexidine

The earliest studies on chlorhexidine mouthwash found it decreases plaque formation and gingivitis (Löe and Schiott 1970). There are several possible mechanisms by which chlorhexidine exerts its plaque inhibitory effects. Chlorhexidine can bind directly to tooth surfaces and prevent adhesion of salivary glycoproteins and subsequent plaque formation. It can also bind to bacterial cell membrane and to pellicle to prevent bacterial adsorption to tooth structures or by disrupting its membrane permeability, or precipitating its cell contents. Finally it can displace calcium ions in plaque films. The plaque inhibitory actions of chlorhexidine may also be a direct effect of its bacteriocidal effects on the bacteria already present, which would prevent their growth. The antimicrobial properties of chlorhexidine, and its ability to adsorb to oral structures appear to facilitate its plaque inhibitory activity. Plaque formation and growth can be controlled by either preventing the proliferation or number of bacteria (Löe and Schiott 1970). The subsequent release of chlorhexidine from oral surfaces is important in maintaining the bacteriostatic environment (Gjermo et al. 1974). There are questions whether the methods of chlorhexidine detection actually differentiate between free molecules or molecules bound to salivary components, bacteria, desquamated epithelium, or other oral debris. Chlorhexidine was detected for longer periods in the saliva than the duration of its bacteriocidal effects, suggesting that the majority of the chlorhexidine in saliva is bound to salivary glycoproteins and is not able to inhibit plaque formation. Plaque inhibitory activity appears to be independent of salivary bacterial reduction.

Plaque inhibition by chlorhexidine has been proposed to decrease the number of bacteria available for adsorption to teeth, blocking the acidic groups on salivary proteins and thus

reducing protein adsorption to teeth, binding to the surface of bacteria to directly interfere with the adsorption of bacteria to teeth or bacterial viability and by precipitating the acidic agglutination factors in saliva and the displacement of calcium which is responsible for the cohesion of plaque (Emilson et al. 1973; Rölla and Melsen 1975). Chlorhexidine prevents plaque accumulation by the binding of the divalent chlorhexidine cation via electrostatic forces to anionic groups on the surface bacteria and salivary protein (Kozlovsky et al. 1994).

In the first instance, the adsorption of chlorhexidine to the cell wall is facilitated by the negative charge of the cell surface. Chlorhexidine lipophilicity is important in its interaction to lipids in the bacterial cell wall (Bonesvoll 1977). Chlorhexidine accesses the cell membrane and, at low concentrations disrupts it causing leakage of intracellular components such as potassium ions and phosphorous containing compounds (Hennessey 1977; Fardal and Turnbull 1986). The internal osmotic pressure can be as high as 30 atmospheres in Gram-positive bacteria and can be 8 atmospheres in the Gram-negative. Therefore when the membrane is disrupted, the steep osmotic gradient between the internal and external bacterial environments would result in a 'forceful' egression of bacterial contents. At high concentrations, the leakage is reduced because precipitation of the cytoplasmic contents occurs. The lethal effects of chlorhexidine are related to the extensive intracellular damage it causes. The precise relationship between the bacteriocidal effects and plaque inhibitory effects of chlorhexidine remain unclear.

The antiseptic effects of chlorhexidine are pronounced against a wide range of gram-positive and gram-negative microorganisms (Bain 1980). The physical attachment of chlorhexidine to bacteria also prevents cell wall repair and cellular reproduction.

Scanning electronmicroscopy studies conclude that chlorhexidine actually does not inhibit bacterial attachment directly, but has a bacteriostatic effect which prevents the proliferation of bacteria (Jenkins et al. 1988). The bacterial plaques on surfaces of specimens appeared to be 'devitalised' by chlorhexidine. It suggests that chlorhexidine has a short term bacteriocidal effect, and the adsorption to pellicle is responsible for the bacteriostatic effects.

Plaque inhibition may be directly due to: the destruction of the transport of sugar in oral streptococci, namely the phosphoenol- pyruvate- phosphotransferase system (Marsh and al 1982), and/or the slow desorption of chlorhexidine. Secondarily, plaque inhibition may be due to immediate short term bacteriocidal effects followed by a bacteriostatic effect that is dependent on the chlorhexidine adsorbed to the pellicle on the tooth surface (Jenkins et al. 1988).

Factors which modify retention of chlorhexidine

The mechanism of bonding of chlorhexidine to oral structures has been of interest because the factors governing retention and subsequent release of chlorhexidine is essential in fulfilling its role as an plaque inhibitory agent (Rölla and Melsen 1975). This binding appears to be affected by the pH, presence of cations and anions of the environment. Up to 30% urea did not displace chlorhexidine bound molecules, it was assumed that hydrogen or hydrophobic bonding also occurs between chlorhexidine and the oral structures. Urea (5M) decreases chlorhexidine retention by about 30%, probably by breaking the weak hydrophobic bonds present (Bonesvoll 1977). Cations, such as barium, calcium and cadmium interfered with chlorhexidine binding in a competitive manner for the anionic binding sites on oral structures. This interference

was most obvious with phosphate groups and moderate with sulphate groups. Cadmium and mercury cations did not effect the binding to sulphate groups. Zinc and magnesium cations had similar effects on chlorhexidine as calcium cations. Calcium cations displaced chlorhexidine binding to carboxyl groups, but not when chlorhexidine was bound to sulphate groups. These observations led to the conclusion that chlorhexidine was firmly bound to acidic ionic exchangers. Calcium (250mM) significantly reduced the retention and increased the release of chlorhexidine (Bonesvoll 1977). Numerous clinical studies have shown the competition between calcium ions and chlorhexidine for binding sites on phosphate groups on the bacterial cell wall, negative carboxyl groups on the mucin layer and sulphate groups on the salivary proteins (Bonesvoll 1977).

The slow release of retained chlorhexidine from oral structures could be attributed to displacement by cations, such as free calcium from newly secreted saliva (Rölla and Melsen 1975). This theory is reinforced by the fact that monovalent cations have little displacing effects on chlorhexidine bound molecules, compared with the effects of divalent cations. This displacement effectively results in a loss of integrity of the membrane and leakage of cell contents and disruption of transportation across the membrane. Calcium cations cannot displace chlorhexidine bound to sulphate groups, but chlorhexidine can displace calcium bound to sulphate groups. This process may be involved in the disruption of calcium bridges involved in maintaining plaque integrity.

The incorporation of chlorhexidine in a toothpaste formula results in an interaction with sodium laurylsulphate which reduces both the retention and plaque inhibitory activity of chlorhexidine (Barkvoll et al. 1989). Chlorhexidine binds to oral tissues, binds to and denatures proteins (Rölla et al. 1970), and it is believed that chlorhexidine and sodium

lauryl sulphate interact to form a salt with low solubility and low antibacterial activity. Hence these two compounds are antagonists and should not be used in the same preparation or within a narrow time-frame. Only when this detergent is used more than 2 hours prior to rinsing with chlorhexidine, then the clinical efficacy of chlorhexidine unaffected.

In an in vitro study, the presence of fluoride dramatically increases the affinity of chlorhexidine for hydroxyapatite (Ben-Yaakov and al 1984).

Summary of factors which modify retention:

- pH below 3.0 decreases chlorhexidine retention;
- up to 30% urea displaced chlorhexidine;
- calcium, zinc, magnesium ions do not displace chlorhexidine bound to sulphate groups;
- calcium, zinc, magnesium ions displaced chlorhexidine bound to carboxyl groups;
- calcium, zinc, magnesium, barium, cadmium ions are in competition with chlorhexidine for phosphate groups;
- cadmium and mercury ions did not effect chlorhexidine binding to sulphate groups;
- fluoride enhances chlorhexidine retention;
- sodium lauryl sulfate interacts with chlorhexidine to form a low solubility salt.

Adverse reactions

Few reports of allergic or irritational reactions to chlorhexidine mouthwashes have been reported (Rushton 1977). Occasional undesirable effects following oral use include: reversible swelling of salivary glands (parotitis); discolouration of teeth, tongue and oral

structures; epithelial desquamation and ulceration of oral mucosa; alterations in taste sensation and unpalatable taste. Synthetic restorations can be stained to a dark brown colour within a week (Bain 1980). These side effects restrict its routine use.

Staining of oral structures is a common undesirable side-effect of chlorhexidine (Leard and Addy 1997). Analytical electron microscopy investigations have shown different compositions of 'non-stained' versus 'heavily stained' plaque scrapings (Warner et al. 1993). The non-stained regions were low in sulphur and metal ions. Heavily stained plaque had high levels of sulphur and metals characterised by amorphous, organic regions which were adjacent to mineralised areas. Mineralised regions were separated from the viable bacterial region by the heavily stained regions. The sulphur concentration in the heavily stained region exhibited an increase by about 40-90mmmol/kg over unstained areas. The iron content in these regions was also shown to increase by 3-4 times. Iron supplementation increased the staining. It is proposed that the staining associated with prolonged use of chlorhexidine is composed of a complex between metals and sulphur-containing organic material. The source of the sulphur may be from salivary lactoferrin (an iron-binding sulphur-containing protein) or bacterial sulphate-binding protein (a sulphur-containing periplasmic binding protein). Chlorhexidine may enhance the incorporation of sulphated proteins into plaque.

A direct relationship exists between staining of oral structures and the frequency of exposure to chlorhexidine (Prayitno and Addy 1979). Staining appears to arise from its adsorption to tooth pellicle and/or plaque as the discoloured pellicle; the discolouration correlates with its plaque inhibitory activity (Prayitno and Addy 1979; Addy et al. 1989). Daily use of a 0.2% chlorhexidine rinse resulted in greater staining than a 0.1% rinse.

There was minimal staining when 0.1% solution was used daily, and this was also less effective in preventing plaque formation (Jenkins et al. 1989). However, *in vitro* staining measured by spectrophotometry of 0.2% and 0.12% chlorhexidine preparations have resulted in similar amount of staining; a 0.1% formula produced less staining but at the expense of some of its plaque inhibitory activity (Addy et al. 1991).

The mechanism of stain formation has been debated for a long time. The correlation between plaque inhibitory activity and discolouration (ie. increased plaque inhibitory activity is found where there is marked staining) suggests that pellicle and not bacterial plaque, are the main sites for extrinsic staining. Research into staining has deduced three possible mechanisms (Addy and Moran 1985; Eriksen et al. 1985; Addy et al. 1991; Warner et al. 1993).

(1) The non-enzymatic browning reaction (or also known as Maillard reactions) (Addy and Moran 1985). The substrates for these reactions are carbohydrates and amino-compounds. These substrates undergo a series of condensation and polymerisation reactions to form melanoidins (a brown pigmented substance). A high pH, surplus amino groups and chlorhexidine catalyse these reaction, whereas sulphur dioxide, sulfites and glucose oxidase inhibits them. The glycoproteins of pellicle (80% protein and 20% carbohydrate) may be a source of substrates for this reaction.

(2) The formation of metal (ie. iron and tin) sulphides occur when the pellicle is denatured by splitting of the disulfide bridges, yielding free sulfhydryl groups. The sulfhydryl groups react directly with these metals to form a brown pigment (Ellingsen et al. 1982). Chlorhexidine is capable of denaturing proteins; the sulphur is available from

exposed thiol groups from denatured protein and the iron may be available from food substances (Ellingsen et al. 1982). The denaturation of the proteins appeared to increase iron adsorption (Nordbo et al. 1983). The stain from chlorhexidine and iron are dose-related; staining increases with large quantities of iron. The denaturation of proteins by the bound chlorhexidine increases iron adsorption dramatically (Fardal and Turnbull 1986). Tea and wine, antibacterial agents and heat from smoking are all strong denaturants (Ellingsen et al. 1982). The reaction between tin and sulphur results in a yellowish pellicle, whereas a brown pellicle results from a reaction between iron and sulphur. Some trivalent and divalent salts, such as iron and tin, could also precipitate dietary substances to produce pigmented complexes (Addy and Moran 1985).

(3) Aldehydes and ketones of food breakdown products. The stain from prolonged use of chlorhexidine appears to be independent of dose, and may have a component of dietary etiology (Fardal and Turnbull 1986). A possible mechanism of staining which has become more popular in recent times is that of precipitation of organic food dyes by chlorhexidine (Addy et al. 1979; Addy and Moran 1985; Addy et al. 1991; Leard and Addy 1997). After exposure to chlorhexidine, pellicle has been shown to be extensively calcified and thickened. Chlorhexidine has been shown to precipitate or bind anionic food dyes to oral surfaces. All coffee brands produced less staining than tea (Leard and Addy 1997). Coffee produced more staining than the negative controls in this study, but were considerably less than the gallic acid derivatives. Tea, red wine and port produced the most rapid and marked staining; the conclusion was drawn that the most chromogenic dietary factors (determined by spectrophotometric analysis), contained gallic acid derivatives (Prayitno and Addy 1979). No staining was evident when chlorhexidine, iron or tea were used alone (Addy and Moran 1985). Brown staining was

produced when tea was used with chlorhexidine. A black stain was observed when tea was used with iron rinses. When tea or coffee were excluded from the diet, chlorhexidine produced significantly less staining. Therefore, the interaction between dietary substances, metals and cationic antiseptics appears to be the major cause of staining. In contrast to earlier findings, Addy suggested that protein denaturation by chlorhexidine to form iron sulfide, does not appear to be the likely mechanism for stain formation (Addy and Moran 1985). There appeared to be a large quantity of iron in the stained material (Nordbo and al 1982). Chlorhexidine has been shown to produce coloured compounds on hydroxyapatite when present with food dyes in the oral cavity. *In vitro* studies have shown that tea and coffee produce staining on specimens which have been exposed to chlorhexidine.

Summary of the factors in stain formation:

- gallic acid derivatives are the most chromogenic dietary factors;
- brown stain is formed when chlorhexidine is used with tea;
- black stain is formed when tea is used with rinses containing iron;
- coffee and smoking resulted in less stain than gallic acid derivatives.

Stain reducers and inhibitors

Studies on stain inhibitors (Ellingsen et al. 1982) reported that zinc salts did not significantly influence the degree of staining at all, although zinc had the potential to form white sulfide when reacted with sulphur. Although stannous fluoride reduces ferric ions to ferrous ions which are then unavailable for sulfide formation (Ellingsen et al. 1982; Fardal and Turnbull 1986), this compound is also a known chromogen. Cuprous and chromous salts also inhibited iron staining by a similar redox reaction. However,

these salts are known chromogens. Oxidising agents remove stains by dissolving the iron sulfide to its soluble sulphate counterpart.

Oxidisers (eg. peroxylmonosulphate) can bleach the staining from chlorhexidine use (Tilliss et al. 1991) by oxidation and formation of sulfites (Eriksen et al. 1985).

Summary of stain inhibitors

- cuprous salts
- chromous salts

Summary of stain reducers

- stannous fluoride
- zinc salts
- oxidisers

Epithelial desquamation

Chlorhexidine may sometimes irritate and damage oral mucosa (Flötra et al. 1971). No clear relationship between the chlorhexidine concentration and the amount of desquamation has been determined. Desquamation may be facilitated by the removal of the protective mucin layer on oral mucosa by precipitation by chlorhexidine. However, the wide variation between individuals to chlorhexidine, may be due to the variations in the amount of phosphates and acidic proteins in saliva.

Unpleasant taste

Unpleasant taste is another distinct adverse side effect of chlorhexidine mouthwash. Therefore, the incorporation of chlorhexidine into a toothpaste formula requires that,

taste needs to be assessed. Alterations in taste sensation following the use of chlorhexidine mouthwash such as hypogeusia and dysgeusia were found to be most prominent for sweet perception, then salty and acidic tastes and lastly bitter (Fardal and Turnbull 1986), in addition to a bitter after-taste and altered taste sensation for prolonged periods (Bain 1980).

Stomatitis/parotits

A rare side effect of long term chlorhexidine use is the development of stomatitis and parotitis (possibly of the viral origin). As chlorhexidine is an antibacterial agent, it would effect the commensal bacteria to a large extent. The stomatitis and parotits may be due to the reduction in commensal bacteria which may in turn favour viral infections, but this has never been proven (Flötra et al. 1971).

2.2. TOOTHPASTES CONTAINING CHLORHEXIDINE

Traditional toothpaste formulations contain humectants, detergents, abrasives, calcium salts, fluoride, preservatives and water (Bonesvoll 1977). In the 1980's, chlorhexidine containing toothpaste and gels were considered to be less effective than the mouthwash preparations (Bain 1980). Very few toothpastes containing chlorhexidine are available, probably because and their plaque inhibitory activity is limited (Binney et al. 1997). The component of toothpastes which limits the efficacy of chlorhexidine is the synthetic detergent. Anionic phosphate ester surfactant (Berol), non-ionic surfactant (Miranol) and Zwitterionic surfactant (Betaine) have all been reported to inactivate chlorhexidine to some extent (Addy et al. 1989). The most commonly used synthetic detergent in toothpaste is sodium dodecyl (lauryl) sulphate, which is usually present in 0.5%-2.0% concentration (Barkvoll et al. 1989). Sodium lauryl sulphate is an effective agent in solubilising proteins bound to biological membranes, and appears to be a major culprit in inactivating the plaque inhibitory effects of chlorhexidine.

The antibacterial activity of chlorhexidine containing dentrifice is not reduced by the addition of fluoride (Dolles et al. 1979), rather the presence of fluoride (sodium monofluorophosphate but not sodium fluoride) increases the affinity of chlorhexidine for hydroxyapatite (Barkvoll et al. 1988). The presence of calcium reduced the retention and increased the release of chlorhexidine (Bonesvoll 1977). A lowered pH reduced the retention of chlorhexidine indicating that electrostatic forces were involved in the adsorption of chlorhexidine to the oral cavity.

There have only been a few long term studies on chlorhexidine containing toothpaste (Eriksen and Gjermo 1973; Johansen et al. 1975; Sanz et al. 1994; Yates et al. 1998). Johansen et al (1975)tested a 0.1% and 0.4% chlorhexidine toothpaste over two years and found there was no reduction in plaque or gingivitis when compared to the control toothpaste. Yates et al (1998) tested 1% chlorhexidine and 1% chlorhexidine/fluoride toothpastes over 6 months; only a small reduction in plaque occurred with these toothpastes when compared with a control toothpaste. Sanz et al (1994) tested a 0.4% chlorhexidine/0.34% zinc toothpaste over 6 months; it reduced plaque accumulation and bleeding sites when compared to the control, but was not as effective as 0.12% chlorhexidine mouthwash. Staining and the use of chlorhexidine toothpaste was correlated in a study in students (Eriksen and Gjermo 1973).

Microbiological investigations

A short term study on the effects on salivary bacterial counts reported that 0.5% chlorhexidine toothpaste did not have any significant reduction in bacterial counts beyond 5 hours (Jenkins et al. 1990). Short term studies of chlorhexidine containing toothpastes on plaque growth have not been conducted.

It is difficult to directly compare studies on the plaque inhibitory effects of chlorhexidine toothpastes tested because of the different concentration of active agents and variable toothpaste base formulations.

2.3. TEA TREE OIL (TTO)

TTO is a naturally occurring antiseptic or antimicrobial agent (Carson and Riley 1994). It is obtained from members of the *Melaleuca* genus. The most common species used is *Melaleuca alternifolia*, and the oil is obtained by steam distillation of the leaves. It generates 1.8% of a pale lemon tint oil which contains 50 to 60% terpenes (pinene, trepinene and cymene) and 6-8% cineol (Altman 1988). TTO comprises over a hundred components (Carson and Riley 1994). Its major antibacterial components are terpinen-4-ol, alpha-terpineol, alpha-pinene and 1,8-cineole (Raman et al. 1995).

Commercial production of TTO began in the 1920s (Carson and Riley 1993). One of the first scientific papers to be published on this antibacterial agent was by Humphery (1930) who introduced a saponified solution of 35% pure TTO which was readily mixed with water. Its first uses included cleansing of open wounds, cuts and abrasions.

In testing eight samples of TTO from different companies against 12 microorganisms, *Pseudomonas aeruginosa* was the only microorganism which was resistant to TTO (Carson and Riley 1994). The microorganisms which were inhibited by TTO included *Escherichia coli, Lactobacillus acidophilius, Staphyloccocus aureus* and *Candida albicans*. Terpinen-4-ol, alpha-terpineol and alpha-pinene were found to have antibacterial activity against *Staphyloccocus aureus, Staphyloccocus epidermidis* and *Propionibacterium acnes*. Cineole was inactive against these microorganisms (Raman et al. 1995). In addition to terpinen-4-ol, the other antibacterial component implicated is cymene (Walsh and Longstaff 1987). However, the presence of cymene was dependent on the location of the plantations. TTO has also been shown to be effective against

Fusobacterium nucleatum, Bacteroides gingivalis, Actinomyces actinomycetemcomitams (Walsh and Longstaff 1987) and oral obligate anaerobes (Shapiro et al. 1994).

A poisoning case documented on a 23 month year old boy who was asymptomatic 5 hours after ingesting 10ml of 100% TTO (Jacobs and Hornfeldt 1994). Undesirable side effects of TTO include skin irritancy (Southwell et al. 1996) in the form of contact dermatitis, mucous membrane irritancy (Walsh and Longstaff 1987) from external use; unconsciousness and general feeling of being unwell from accidental ingestion of concentrated TTO (Carson and Riley 1995).

The therapeutic uses of TTO include acne, aphthous stomatitis, burns, herpes, insect bites, thrush, tonsilitis, tinea (Tong et al. 1992), periodontitis (Walsh and Longstaff 1987) and gingivitis. Few clinical trials investigated the effectiveness of TTO as an oral hygiene product. A recent *in vitro* study on the preparations tested in Parts 2 and 3 of this study, concluded that TTO has potential as an antimicrobial agent in mouthrinses (Rogers and Gully 1999).

2.4. INDICES

PLAQUE ACCUMULATION INDICES

Numerous methods have been used to measure plaque growth on teeth (Ramfjord 1959; Greene and Vermillion 1960; Quigley and Hein 1962; Silness and Löe 1964; Turesky et al. 1970; Stean and Forward 1980; Mombelli et al. 1987; Addy et al. 1998)

The Record of Plaque accumulation was probably the first index of its kind (Ramfjord 1959).

Table 2.1 Ramfjord (1959): Record plaque accumulation

P0	no plaque present
P1	Plaque present on some but not all of the interproximal and gingival surfaces of the tooth
P2	Plaque present on all interproximal and gingival surfaces but covering less than one half of the entire clinical crown
P3	Plaque extending over all interproximal and gingival surfaces covering more than one half of the entire clinical crown

Ramjford made the point that disclosing solution needed to be used because the similarities in colour of plaque and enamel contribute significantly to measurement error. The index also took the interproximal plaque into account. However, recordings using only Ramjford teeth (16, 21, 24, 36, 41, 44) result in missing data, and may not reflect the overall plaque accumulation in an individual. This index in its pure form was therefore not appropriate for use in this research project. However, the index could have been used to score all the teeth.

Greene and Vermillion (1960) devised the Oral Debris Index (ODI) which required examination of the buccal and lingual surfaces of all teeth, resulting in 2 scores to be

given to each sextant, one for the buccal, and one for the lingual surface (Greene and Vermillion 1960).

Table 2.2 Greene and Vermillion (1960): Oral debris index

0	no debris or stain present
1	soft debris covering < one third of tooth, OR stain present
2	soft debris covering >one third, < two thirds of tooth
3	soft debris covering > two thirds of tooth

Only the tooth surface with the most oral debris was scored in each sextant. The definition of plaque accumulation according to coverage of tooth crown is easy to apply in the clinical setting, and removes the subjective component in scoring.

One can also appreciate that a lot of data are lost when only the most debris for a sextant is scored. Perhaps scoring of individual tooth surfaces initially, followed by analysis of sextants or groupings of teeth would better reflect the pattern of plaque accumulation. The definitions of the scores are also of concern, as stain is included in both scores 0 and 1. The stain component should be scored separately, as the origin and occurrence of stain is not the same as plaque. This index in its pure form was not appropriate for this project.

The plaque index chosen is the Plaque Scoring System (PSS) (Quigley and Hein 1962), which measured plaque accumulation relative to the coverage of the crowns of the anterior teeth. The definitions are detailed and appear to be easy to use.

This research project will use the PSS by Quigley and Hein and will extend its use to the posterior teeth.

Table 2.3 Quigley and Hein (1962): Plaque scoring system

0	no plaque
1	flecks of plaque at gingival margin
2	definite line of plaque at gingival margin
3	gingival third of surfaces
4	two thirds of surface
5	> two thirds of surface

The plaque index which was one of the first widely used established indices was the Plaque Index (PI) (Silness and Löe 1964).

Table 2.4 Silness and Löe (1964): Plaque index

0	no plaque
1	a film of plaque adhering to the free gingival margin and adjacent area of the tooth, plaque may be seen in situ only after application of disclosing solution or by probing
2	moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eye
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

This index was the first of its kind to objectively quantify the amount of plaque accumulation on the buccal/labial and palatal/lingual surfaces of six representative teeth (16, 12, 24, 36, 32, 44). The index was quick to use and allowed ease of comparison of data by using specific teeth. However, this index resulted in loss of information because analysis of the data could not reliably be made on different groups of teeth (ie. anterior versus posterior types of teeth). The scale of 0-3 was a useful quantifying tool, but the definitions of each category were vague. For example, how did an operator interpret the term 'moderate' and 'abundance'? Although easy and quick to use, the definitions of PI may be open to interpretation.

The Quigley and Hein index (Quigley and Hein 1962) measured area of plaque present in relation to the crown of a tooth, and was modified by Turesky in 1970 (Turesky et al. 1970). Turesky's modification appears to be the addition of numerical limits to the Quigley and Hein index.

Table 2.5 Turesky et al (1970): Plaque index

0	no plaque
1	flecks of plaque at gingival margin
2	definite line of plaque at gingival margin <1mm
3	< gingival third of surfaces >1mm
4	two thirds of surface
5	two thirds of surface

An attempt to remove subjectivity from the measurement of plaque resulted in the development of the Plaque Area index (PAI) (Stean and Forward 1980).

Table 2.6 Stean and Forward (1980): Plaque area index

surfaces measured - labial surfaces of first molars and all teeth anteriorly
only assessed plaque attached to gingiva
disregarded unattached plaque and pellicle
plaque area in millimetres squared obtained from Electronic area measuring unit, from drawings of plaque

This index involves the measurement of the area of the labial surfaces of all available incisors, canines, premolars, and first molars. The plaque area attached to the gingival margin was drawn on a tooth chart. Unattached plaque and pellicle were not considered. The areas of plaque on each tooth were digitised and processed by a computer. The requirement for technical equipment can lead to a very expensive initial outlay. This index is expensive and may be labour intensive.

In 1987, a modified Plaque Index (mPI) was developed (Mombelli et al. 1987).

Table 2.7 Mombelli et al (1987): mPlI (modified plaque index)

0	no detection of plaque
1	plaque only recognised by running probe along smooth surface of implant
2	plaque seen by naked eye
3	abundance of soft matter

This index used the numerical score similar to the PI, but the score definitions varied. Mombelli et al (1987) had developed this index to measure plaque on implants. Plaque can be easier seen on the metal surface of an implant than it would be on the enamel on the tooth. Taking that into account, and extrapolating the use of this index to teeth, the index would still be easier to apply than the PI. The mPI score 0,1, and 2 are straight forward to apply in the clinical setting. However, the mPI score 3 is still open to interpretation; where the plaque score ends at 2 and where it becomes 3 is difficult to standardise between operators.

The possibility of measuring plaque on every tooth surface was considered. The Occlusal Plaque Index (OPI) (Addy et al. 1998).

Table 2.8 Addy et al (1998): Occlusal plaque index

0	no disclosed plaque or discrete flecks in fissure pattern
1	line of plaque in fissure pattern but not outlining whole fissure system
2	fissure system completely outlined by plaque
3	plaque beginning to extend out of the fissures, at some sites with <1/3 coverage
4	plaque extending out of the fissure system with 1/3 to 2/3 coverage
5	plaque extending to cover >2/3 of occlusal surfaces

The Addy et al (1998) index was a modification of the Shaw and Murray 1977 index. This index was only useful in posterior teeth, and needed to be used in conjunction with

another plaque index. In addition, the delicate nature of plaque may easily be dislodged on occlusal surfaces, and heavily dependent on what the last meal was. Hence, measuring the plaque accumulation on the occlusal surfaces had its limitation.

Summary of Plaque Indices

The plaque index by Quigley and Hein (1962) was chosen for this study. The plaque area index may seem attractive due to its mathematical simplicity and objectiveness, but it was too time consuming and tedious to use, especially without computerised support. In addition, the plaque indices have been shown to have greater discriminatory power compared to plaque area indices in most studies reviewed (Addy et al. 1999). This means that, for example the Turesky index (1970), is better able to discriminate between high and low plaque formers compared to the plaque area index, a modification of Shaw and Murray's grid method for assessment of plaque area (Shaw and Murray 1977).

GINGIVAL HEALTH INDICES

Gingival health can be assessed on the degree of inflammation, the amount of bleeding on probing and degree of change in texture and contour from normal.

The gingival health index by Ramfjord in 1959 was probably the first to be devised and it used only six 'Ramfjord' teeth (16, 12, 24, 36, 41, 44).

Table 2.9 Ramfjord (1959): Record of gingival health (of 16, 21, 24, 36, 41, 44)

G0	absence of inflammation
G1	mild to moderate inflammatory gingival changes are extending all around the tooth
G2	mild to moderately severe gingivitis extending all around the tooth
G3	severe gingivitis characterised by marked redness, tendency to bleed, ulceration

The definitions of the scores consist of two elements: the extent to which the gingiva around a tooth was affected is only considered if it surrounds the entire tooth and the amount of inflammatory change which is present. The interpretation of mild, moderate and severe gingivitis is subjective, and this introduces operator error. In addition, it is not clear what score should be given if the gingival changes are not uniform around a tooth. How are non-ulcerated bleeding gingiva scored? Again, the lower end of the scores are too narrow because they do not allow for subtle changes in gingiva to be recorded independently. This index was a good first attempt to assign a numerical value to gingival health but was unsuitable for use in this study because of its ambiguity in definitions, and the narrowness of the score definitions.

In 1963, Löe and Silness described their Gingival Index (Löe and Silness 1963) utilising the Ramfjord teeth.

Table 2.10 Löe and Silness (1963): Gingival Index (Ramfjord teeth)

0	absence of inflammation
1	mild inflammation - slight change in colour and little change in texture
2	moderate inflammation - moderate glazing, redness, edema and hypertrophy
3	severe inflammation - marked redness, hypertrophy, tendency to spontaneous bleeding, ulceration

In addition, they developed a scoring system for the 4 surfaces of each tooth and slightly expanded the definitions. The score for each tooth was obtained by adding the scores of the 4 tooth surfaces and dividing that by 4. From there, scores could be grouped according to types of teeth under consideration. The division of the gingiva into 4 corresponds with the 4 tooth surfaces, and allowed the different degrees of gingival health to be expressed around a single tooth. However, since there were only 2 representatives of each tooth type, the extrapolation of scores toward a generalised statement about that group of teeth was probably neither accurate nor reliable. In addition, the definitions of the scores 2 and 3, are too severe and would not be of much use in a research project such as this. In fact, they would not apply to gingiva in people practising some form of oral hygiene practices. Hence, the scores need to be expanded in the lower end to measure subtle changes in the gingiva.

In 1967, Löe modified the Gingival Index (Löe 1967) to apply to all teeth (ie. not just the 6 Ramfjord teeth).

Table 2.11 Löe (1967): Gingival Index (all teeth)

0	normal gingiva
1	mild inflammation, slight change in colour, slight edema, no bleeding on probing
2	moderate inflammation, redness, edema, glazing, bleeding on probing
3	severe inflammation, marked redness and edema, ulceration, tendency to spontaneous bleeding

Scores could be assigned for individual surfaces, teeth, groups of teeth and the individual person. The revised index addressed the shortcomings of the indices developed before it, and became the 'standard' index for many years. However, the shortfalls of this index continued to be the narrowness of the lower end of the scores, and the definitions of moderate and severe inflammation. The definitions of the scores reflected gingival conditions which were far too advanced for the observations of the present research project and would result in clumping of scores at the lower end. Subtle gingival changes cannot be accurately reflected in the scores. However, this index was chosen for this project because it is still considered to be the 'standard index' for gingival health; it would allow comparisons to be made with other studies which also used this index.

The most simple indices measure gingival health by recording the absence or presence of bleeding after probing (Carter and Barnes 1974; Ainamo and Bay 1975; Velden 1979; Abrams et al. 1984). Quite a few authors utilise this system, using various locations of probing, specifying probing force and time taken for bleeding to occur.

The absence of bleeding is a negative predictor of disease. The presence of bleeding provides better information on gingival health status than gingival colour.

Table 2.12 Carter and Barnes (1974): Gingival Bleeding Index

unwaxed floss used - 2 movements inciso-gingivally
sextants
absence & presence of bleeding only
mesial and distal sulci are scored as one interdental unit
third molars are not scored
initial & subsequent 30sec bleeding
total scoreable, total bleeding, total non bleeding
Score obtained by total bleeding/total susceptible sites

Table 2.13 Ainamo and Bay (1975) Gingival Bleeding Index = site prevalence index

Blunt probe used to probe gingival crevice, no pain induced
Bleeding seen < 10secs = positive finding recorded
number of positive sites expressed as % of number of gingival margins examined

Table 2.14 Velden (1979): PPBI - periodontal pocket bleeding index

0	no bleeding of the pocket after probing with force 0.75N
1	bleeding of pocket within 30 secs after probing with force 0.75N

Table 2.15 Abrams et al (1984): Bleeding index

Wooden interdental cleaner inserted interdentally to depress papilla 2mm
presence or absence of bleeding within 15 seconds

Later indices became more complex and assigned scores to the degrees of inflammation of the gingiva (Mühlemann and Son 1971; De La Rosa and Sturzenberger 1976; Lobene et al. 1986). The concept of the extent of gingival inflammation itself is sound in terms of measuring gingival health. However, the definitions of these scores were not appropriate to describe the majority of gingival tissues we were going to observe in the evaluation of oral health care products. These definitions would probably be more appropriate in 'dentally neglected cases'. For example, glazing, edema, hypertrophy, spontaneous bleeding and ulceration of gingival tissues is highly unlikely in individuals

with some form of oral hygiene and individuals who presented with these signs would not have been included in the study.

Table 2.16 Mühlemann and Son (1971): Gingival sulcus bleeding

0	absence of inflammation
1	mild inflammation, slight change in colour, little change in texture, no bleeding on probing
2	moderate inflammation, moderate glazing, redness, edema, hypertrophy, BOP
3	severe inflammation, marked redeness, hypertrophy, tendency to spontaneous bleeding

Table 2.17 De La Rosa and Sturzenberger (1976):

PMGI (papillary marginal gingivitis index)

0	no inflammation, normal gingiva
1	mild inflammation, slight change in colour and little change in texture
2	moderate inflammation, moderate glazing, redness, edema, enlargement, bleeding on pressure
3	severe inflammation, marked redness, enlargement, tendency to spontaneous bleeding, ulceration

Table 2.18 Lobene(1986): A modified gingival index from(GI of Loe and Silness)

0	absence of inflammation
1	mild inflammation, slight change in colour and little change in texture
2	moderate inflammation, moderate glazing, redness, edema, hypertrophy, bleeding on pressure
3	severe inflammation, marked redness, hypertrophy, tendency to spontaneous bleeding, ulceration

The Sulcus Bleeding Index (SBI) expanded the lower range of the scores, but used definitions such as 'colour change' to distinguish between the different degrees of inflammation (Mühlemann and Son 1971).

Table 2.19 Mühlemann and Son (1971): Sulcus Bleeding Index (SBI)

1	facial M units
2	mesial P units
3	distal P units

0	healthy appearance of P & M, not bleeding on sulcus probing
1	apparently healthy P & M showing no change in colour and no swelling, but bleeding form sulcus on probing
2	bleeding on probing and change in colour due to inflammation, no swelling or macroscopic edema
3	bleeding on probing and change in colour and slight edematous swelling
4	bleeding on probing, change in colour, obvious swelling
5	bleeding on probing, spontaneous bleeding, change in colour, marked swelling with or without ulceration

The colour change at the lower end of the scoring range is difficult to apply clinically, and only severe cases of gingival inflammation would display colour change. The other difficulty is how the operator is to determine what caused the colour change. It is not clear how an operator should distinguish between slight edematous swelling and obvious swelling. The difficulty in applying this index clinically limits its use. As it is open to interpretation, the index itself introduces inconsistencies between observations, between operators, and decreases the reproducibility of data.

The Papillary Bleeding Index (PBI) (Newbrun 1996) introduced by Saxer in 1975, added the dimension of time into measuring gingival health, as seen by the time taken for bleeding to occur after probing.

Table 2.20 Saxer (1975) - (summary from Newbrun 1996):

PBI papillary bleeding index

0	no bleeding within 30sec of probing
1	bleeding within a few seconds of probing
2	immediate bleeding on probing
3	bleeding along gingival sulcus on slightest touch

Table 2.21 Saxer (1977) - (summary from Newbrun):

PBI papillary bleeding index - revised

0	no bleeding
1	single bleeding point 20-30sec after probing
2	fine line of blood or several bleeding points
3	blood fills interdental triangle soon after probing
4	immediate profuse bleeding, fills interdental area, flows over tooth & gingiva

This was further refined to associate time with the amount, in terms of pattern of bleeding. This index provides objective definitions to facilitate uniformed scoring by operators, and incorporates the time factor.

In a parallel development, Mühlemann introduced the Papillary Bleeding Index (PBI) without the time component (Fischman 1988).

Table 2.22 Mühlemann (1977) - (summary from Fischman 1988):

Papillary bleeding index (PBI) - probing of interdental papilla

0	no bleeding
1	only one bleeding point present
2	several isolated bleeding points or a small area of blood
3	interdental triangle filled with blood
4	profuse bleeding spreading toward the marginal gingiva

This form of the PBI was objective in its definitions and could be a reliable index by increasing the reproducibility of scoring. To add to the reliability of this index, the present research project used manual pressure sensitive probes (using a force of 20 grams) to probe the interdental papilla, thereby standardising the probing force.

The Papillary Bleeding Score (PBS) was determined on all papillae anterior to the second molars and omits readings from the buccal and lingual gingival margins (Loesche 1979).

Table 2.23 Loesche (1979): Papillary bleeding score-compared with gingivitis index

PBS = 0	GI = 0	healthy gingiva, no bleeding upon insertion of Stimudent interproximally
PBS = 1	GI = 1	edematous, reddened gingiva, no bleeding upon insertion of Stimudent
PBS = 2	GI = 2	bleeding without flow upon insertion of Stimudent
PBS = 3	GI = 2	bleeding with flow along gingival margin upon insertion of Stimudent
PBS = 4	GI = 2	copious bleeding upon insertion of Stimudent
PBS = 5	GI = 3	severe inflammation, marked redness & edema, tendency to spontaneous bleeding

The PBS expanded the GI score 2 into 3 easily recognisable clinical observations to address the lower end of the scores and thereby facilitated clinical application of the index. In effect, the PBS resembled the SBI, but used Stimudent instead of a probe; variation in insertion of the Stimudent may also be of concern here, causing inconsistencies in observation. Where this index differs from the other gingival bleeding indices (Carter and Barnes 1974; Ainamo and Bay 1975; Velden 1979; Abrams et al. 1984), is that the PBS is concerned with the presence or absence of bleeding and the pattern of bleeding when it occurs, whereas the former were only concerned with the absence or presence of bleeding.

The Gingival Bleeding Time Index (GBTI) introduced time as another parameter when measuring gingival health (Nowicki et al. 1981).

Table 2.24 Nowicki et al (1981): Gingival bleeding time index

- probe inserted into sulcus until resistance felt - moved 2mm back & forth

- score 2 for bleeding < 15secs

0	no bleeding within 15 secs of twice probing
1	bleeding within 6 - 15 secs of second probing
2	bleeding within 11 - 15 secs of first probing OR 5 secs after second probing
3	bleeding within 10 secs after initial probing
4	spontaneous bleeding

However, it did not account for the variable of probing pressure. This index is time consuming, as the gingiva around each tooth are required to be probed individually and the appearance of bleeding timed, before proceeding to the next tooth. This index was impractical for use in this study.

The Modified Gingival Index (MGI) was developed to overcome the problems inherent in the earlier indices (Lobene et al. 1986).

Table 2.25 Lobene et al (1986): A modified gingival index MGI

0	absence of inflammation
1	mild inflammation, slight change in colour, little change in texture of any portion of but not the entire marginal or papillary gingival unit
2	mild inflammation, criteria as above but involving the entire marginal or papillary unit
3	moderate inflammation, glazing, redness, edema, +/- hypertrophy of the marginal or papillary gingival unit
4	severe inflammation, marked redness, edema +/- hypertrophy of marginal papillary gingival unit, spontaneous bleeding, congestion, ulceration

When compared to the Gingival Index, the MGI eliminated the use of pressure, redefined the definitions of mild and moderate inflammation, with the score of 1 for partial inflammation of gingival tissue around a tooth, and a score 2 for inflammation of all of the gingiva surrounding a tooth. Higher scores of 3 and 4 were assigned for more

severe inflammation. The MGI expanded the lower end of the scoring range of the Gingival index.

The Modified Bleeding Index (mBI) used around implants (Mombelli et al. 1987), can also be applied to natural teeth but interproximal contacts limited the access to the circumference of teeth.

Table 2.26 Mombelli et al (1987): mBI (modified bleeding index)

0	no bleeding when periodontal probe is passed along gingival margin adjacent to implant
1	isolated bleeding spots visible
2	blood forms a confluent red line on margin
3	heavy or profuse bleeding

Summary of Gingival Health Indices

The indices used to assess gingival health in this study were the Gingival Index by Löe in 1967 (Löe 1967), and the Papillary Bleeding Index (PBI) by Mühlemann (Fischman 1988). The first index was used because it provides a standardised index to compare with other similar studies. Admittedly, the concerns regarding this index do limit its value. The PBI, on the other hand is more appropriate for this study as an objective scoring system.

STAIN INDICES

One of the aims of this project was to measure the amount of staining on teeth, and the tongue.

The variables associated with staining are coverage, intensity and distribution. Staining indices, as with the plaque indices, ranged from the subjective definitions of 'noticeable' to 'obvious'; to the grid square index where each tooth surface was divided into over 400 squares. The following four indices addressed the severity of staining, using definitions such as 'slight', 'light' to 'severe', 'heavy' staining (Prayitno et al. 1979; Addy and Moran 1985; Addy et al. 1991; Soskolne et al. 1997). These definitions may be open to interpretation and were not used in this project.

Table 2.27 Prayitno et al (1979): Severity of staining

0	no stain
1	slight stain
2	moderate stain
3	severe stain
4	very severe stain

Table 2.28 Addy and Moran (1985):

Extrinsic tooth discolouration (on anterior teeth)

0	no change from baseline
1	just noticeable
2	obvious
3	very apparent

Table 2.29 Addy et al (1991): Visual stain score

0	no stain
1	very slight stain
2	slight stain
3	moderate stain
4	heavy stain

Table 2.30 Soskolne et al (1997): Supragingival stain index

0	no detectable stain
1	slight stain
2	moderate stain
3	severe stain

In 1994, Sanz et al introduced a staining index which addressed the overall characteristic of stain, intensity and coverage of the stain.

Table 2.31 Sanz et al (1994):

Overall stain (0-6)	0 = no staining 6 = very dark stain
Intensity (0-4)	0 = no discolouration 4 = very dark stain
Coverage (0-6)	0 = no coverage 6 = > 30% coverage

This is the most detailed stain index to date as it addressed overall appearance of the stain, its intensity and coverage. However, the definitions of the overall appearance and intensity components may be open to operator interpretation. The degrees of 'darkness' in the score definition can vary greatly. Perhaps it would have been better to address the 'overall' appearance of the stain to colours, such as yellow, brown and black. The coverage component was objective, but required careful measuring, as the score of 0-6 ranged over 30% coverage, hence each increment in the score accounted for 5% of coverage by stain.

Shaw introduced a new index for measuring extrinsic staining which used mainly objective physical measurements (Shaw and Murray 1977).

Table 2.32 Shaw and Murray (1977): Grid method

4mm grid method of the labial & lingual of 8 incisors scaled drawings x4 magnification, each tooth divided into 4mm squares 412 sq on La, 422 sq on Li surfaces

This index used scanned, standardised photos and computer programs to assist in the calculations of the amount of stain present on the labial and lingual/palatal surfaces on the 8 anterior teeth. Each tooth's labial surface was divided into 412 squares and on the lingual, into 422 squares by superimposition of a grid on to standardised photographs. The area of staining was analysed in terms of the number of squares covered in stain. This method is numerically accurate, but extremely time and labour intensive.

The intensity and amount of staining were addressed independently by Tilliss' indices (Tilliss et al. 1991).

Table 2.33 Tilliss et al (1991): (modified from Lang & Raber, Lang & Hotz)

Stain Intensity grading

0	no stain
1	light stain, barely visible light yellow to brown
2	medium stain, visible medium brown colour
3	dark stain, dark brown to black colour

Stain amount grading

0	no staining
1	thin line of stain (<1mm in width)
2	moderate band of stain (1-2mm in width)
3	wide band of stain (>2mm width)

However, this index only applied to the labial surface of teeth (ie. the disto labial, labial, mesiolabial surface). With the use of a mouthwash, the solution would probably be in

contact with the mandibular lingual surfaces of teeth for the longest period, compared to any other tooth surface. Hence, taking the readings from the labial surfaces only may result in a skewed incidence of staining, as the areas measured were not necessarily the areas where maximum contact with the solution occurs. The other consideration is that perhaps the staining on labial surfaces is the only staining which is aesthetically important; ie. is there a need to measure staining where it does not effect aesthetics? This index in its pure form was not used in this research project because it did not measure staining on all surfaces of teeth. However, an index can be modified to score any amount of teeth.

The stain index chosen for this project was the Discolouration Index System (DIS) which measured stain on the buccal and lingual surfaces (Lang and Raber 1981).

Table 2.34 Lang and Raber (1981): Discolouration Index system

0	no discolouration, clean polished tooth surface, natural appearance in colour
1	slight yellow discolouration, yellowish film over the entire extent of the clinical crown, slight brownish discolouration along the gingival margin
2	moderate brownish discolouration on the interproximal surfaces and in the apical third of the clinical crown
3	heavy, brown and black discolouration over the entire extent of the tooth surface, black discolouration predominantly on the interproximal surfaces

The definitions of the scores addressed the degree (ie. the colour gradings) and the extent of coverage of the tooth crown. The definitions are objective and minimise intra-operator inconsistencies.

All the stain indices discussed to this point refer to the hard tissues of the oral cavity. In order to assess the staining effects on the soft tissues, the tongue was chosen as it is in a position of maximum exposure to substances placed in the mouth. The following index which addresses the amount of coverage of tongue by stain was chosen (Prayitno et al. 1979).

Table 2.35 Prayitno et al (1979):

Tongue dorsum - % of total area of dorsum covered by stain

1	25% coverage
2	50% coverage
3	75% coverage
4	100% coverage

The scores vary according to the percentage of the tongue dorsum coverage by staining. This was considered to be objective, and was thought to result in the minimal amount of intra-operator inconsistencies. This index was used in the present study.

The Discolouration Index System (DIS) which measured stain on the buccal and lingual surfaces by Lang and Raber 1981 was used in this study. It is an objective and simple index.

Summary of hypotheses

In summary, this thesis is concerned with reporting on the clinical trials involving two test preparations, namely:

- chlorhexidine containing toothpaste, and
- tea tree oil mouthwash.

The plaque inhibitory activity of chlorhexidine toothpaste was tested in a randomised crossover blind 4 day plaque growth model, against 0.12% chlorhexidine mouthwash, Colgate Total® and the chlorhexidine toothpaste base. The hypothesis was:

H_o 1: There is no difference between chlorhexidine toothpaste and 0.12% chlorhexidine mouthwash in their plaque inhibitory action.

The plaque inhibitory activity of TTO mouthwash was tested in a randomised crossover blind 4 day plaque growth model, against 0.12% chlorhexidine mouthwash, Listerine® and a mouthwash base. The hypothesis was:

H_o 2: There is no difference between tea tree oil mouthwash and 0.12% chlorhexidine mouthwash in their plaque inhibitory action.

The anti-plaque action of TTO mouthwash was tested in a randomised blind 6 week study, against a placebo mouthwash base. The hypothesis was:

H_o 3: There is no difference between tea tree oil mouthwash and placebo mouthwash in their effect on gingival health.

Chapter 3

MATERIALS AND METHOD

3.1 CLINICAL CONSIDERATIONS

4 DAY PLAQUE GROWTH MODEL

Short term studies of variable duration have been used to assess plaque regrowth; these have ranged from as short as 16 hours to 4 days. In assessing the efficacy of oral hygiene products in the prevention of plaque accumulation, the 4 day plaque regrowth model is preferred and was used in this study (Sjöblom et al. 1976; Addy et al. 1983; Addy et al. 1989; Jenkins et al. 1989; Binney et al. 1992; Moran et al. 1992; Rundergren et al. 1992; Jenkins et al. 1993; Moran et al. 1994; Jenkins et al. 1994a; Jenkins et al. 1994b; Binney et al. 1995; Moran et al. 1995; Smith et al. 1995; Binney et al. 1996; Renton-Harper et al. 1996; Binney et al. 1997). The model overcomes the toothbrushing variable by removing it. That is, the variation in brushing techniques and efficiencies between subjects do not have to be considered in the analysis. Plaque accumulation over 4 days, in the absence of mechanical plaque removal, provides enough time for sufficient plaque to accumulate to facilitate ease of plaque assessment, without excessive plaque sloughing off. The 4 day plaque growth model was described in detail by Addy (Addy et al. 1983) and is characterised by the following. At bascline (day 0), the subjects receive a scale and clean, and prophylaxis to remove all plaque. The subjects rinse twice daily with the mouthrinse (be it a mouthwash or a toothpaste slurry) over a 96 hour period. No mechanical oral hygiene practices are used during this period. At the end of the 4 day period, the subjects' teeth are stained with a plaque

disclosing solution and plaque is scored. Plaque is then removed by scaling and dental prophylaxis and subjects resume their normal mechanical oral hygiene practices.

Toothpaste slurries

In order to test the antiplaque effects of an oral hygiene product, a toothpaste slurry was used (Sjöblom et al. 1976; Addy et al. 1983; Addy et al. 1989; Jenkins et al. 1989; Binney et al. 1992; Moran et al. 1992; Binney et al. 1995; Binney et al. 1996; Binney et al. 1997). A length of toothpaste was mixed into slurry prior to the subjects rinsing. The protocol for mixing (which involves stirring and shaking) the toothpaste strip and the liquid medium into a toothpaste suspension can be standardised. The advantage of using the toothpaste slurry is that the plaque inhibitory effects of the toothpaste can be evaluated in the absence of toothbrushing.

Crossover / randomised study design

A crossover design (Sjöblom et al. 1976; Addy et al. 1983; Addy et al. 1989; Jenkins et al. 1989; Binney et al. 1992; Moran et al. 1992; Rundergren et al. 1992; Jenkins et al. 1993; Moran et al. 1994; Jenkins et al. 1994a; Jenkins et al. 1994b; Binney et al. 1995; Moran et al. 1995; Smith et al. 1995; Binney et al. 1996; Renton-Harper et al. 1996; Binney et al. 1997) is usually employed to assess plaque growth. The advantages of using a crossover study are the need for fewer subjects and that each subject is their own control. For example, when four preparations are being tested, only one quarter the number of subjects is required compared with a parallel design for equivalent statistical powers. Each individual is their own control as the physiological and physical aspects of the oral cavity remain relatively unchanged within the individual. An individual with a crowded dentition may be more predisposed to plaque formation compared to someone

with a perfect occlusion. Regardless of the arrangement of the dentition, the amount and pattern of plaque formation for one person is probably relatively consistent for that person. The rate and pattern of plaque formation is less likely to vary in the same individual, while there are statistically significant differences in these parameters between individuals. A crossover study, where each subject is their own control, has a greater statistical power to detect differences in a preparation, as opposed to a parallel study. As there were four mouthrinses involved in both Parts 1 and 2 of this trial, the study needed to be randomised, and balanced for carryover effects; when there are three or more preparations, designs that balance for first order carryover would have each formula preceded by each of the others in the same number of subjects (Newcombe et al. 1995). This should apply for all the four mouthrinses and for every position in the sequence of testing. However, the perfectly balanced study may not always be attainable without a prohibitively large number of subjects and unforseen drop-out of subjects. Adjustments to the data may minimise the imbalance from drop-outs. Randomisation of treatments also increases the validity of the results of a clinical trial. If the preparations were issued in the same order for all subjects, there is serious bias towards the last preparation, the results of which may have some cumulative effects of the preceding preparations. In addition, the investigator may inadvertently issue the non-test preparation to subjects who may be embarking on a 'high sucrose' period (such as during the Easter festive season). Randomisation protects against such bias, and chance alone determines which preparation is issued to which subjects. The preparations were packaged in identical rectangular white boxes, which were coded. All the preparations were issued by the same investigator who scored the teeth at the review appointments. It is not ideal to have the same person issue the preparations and review the subjects, but there was limited resources available resulting in no alternative in the

present study. All preparations were issued in coded identical rectangular boxes. The code breaker was not revealed to the investigator until after all the data had been collected and analysed.

Double-blind

To avoid any inadvertent bias in the use or the assessment of the mouthrinses, a double-blind design (Addy et al. 1989; Moran et al. 1992; Rundergren et al. 1992; Jenkins et al. 1993; Moran et al. 1994; Jenkins et al. 1994a; Jenkins et al. 1994b; Smith et al. 1995) is required, where both the assessor and the subjects are unaware of the precise contents of the preparations being assessed. The toothpaste preparations were tested in a double blind setup; and the chlorhexidine mouthwash was tested in a single blind setup (that is blind to the investigator).

Residual effects

Chlorhexidine gluconate has been used as the 'gold standard' the positive control in many studies evaluating the plaque inhibitory efficacy of different agents. This product has been generally accepted as the most efficacious plaque inhibitory agent to date (Löe and Schiott 1970). Chlorhexidine retention in the oral cavity has been detected for at least seven hours after use. This substantivity is closely related to its positive attribute of plaque inhibitory activity, but this may pose a problem with carry-over or residual effects (Newcombe et al. 1995). Thus, the consideration of washout periods is important.

Washout period

The washout periods between preparations in a crossover clinical trial vary between studies, and range from 48 hours (Addy et al. 1989; Moran et al. 1994) to 24 days (Rundergren et al. 1992). The majority of the studies have been designed around a 2.5 hour to 3 day washout period. The common positive control in these studies is chlorhexidine mouthwash. A comparison of the residual effects of chlorhexidine against inert negative controls such as water or saline (Newcombe et al. 1995) concluded that a washout period of 10 days or greater is preferable. Designs should be balanced for residual effects of the preceding treatment. In this clinical trial, there was at least a 16 day washout period between the testing periods; the washout period ranged from 16 to 45 days.

Rinsing times, duration and amount

The volume of mouthwash or slurry used in plaque growth studies range from 10ml (Addy et al. 1983; Addy et al. 1989; Jenkins et al. 1989; Binney et al. 1992; Moran et al. 1992; Rundergren et al. 1992; Jenkins et al. 1993; Moran et al. 1994; Jenkins et al. 1994a; Jenkins et al. 1994b; Moran et al. 1995; Smith et al. 1995; Binney et al. 1996; Binney et al. 1997) to 20ml (Moran et al. 1994). The rinsing is usually performed twice a day, with a duration from 30 seconds (Moran et al. 1994; Moran et al. 1995) to 60 seconds (Addy et al. 1983; Addy et al. 1989; Jenkins et al. 1989; Moran et al. 1992; Rundergren et al. 1992; Jenkins et al. 1993; Moran et al. 1994; Jenkins et al. 1994a; Jenkins et al. 1994b; Binney et al. 1995; Moran et al. 1995; Smith et al. 1995; Binney et al. 1996; Renton-Harper et al. 1996; Binney et al. 1997) .

The study by Cumming and Löe (1973) showed that larger volumes of 400-700 ml of chlorhexidine prevented plaque formation on all surfaces (Cumming and Löe 1973). Good levels of oral hygiene were achieved with 50 and 200ml volumes, but there was a tendency for plaque to develop on some surfaces of posterior teeth. The 20ml volume displayed poor plaque control. The most effective duration of rinse which was found to be 60 seconds. The full 60 seconds allowed time for the chlorhexidine to spread in the oral cavity and increased the probability that all surfaces were in contact with it. Volumes of 50ml and greater resulted in greater effectiveness in plaque control because they required multiple rinsings. Multiple rinsing increases the time the solution is present in the mouth, which in turn increases the chance of it contacting all tooth surfaces. There appears to be no increase in effectiveness with volumes over 100ml. The most commonly used clinical regimen for chlorhexidine is a twice-daily, one minute rinse with 10ml of a 0.2% chlorhexidine gluconate solution (Addy et al. 1989).

TTO 6 WEEK EFFECTS ON ORAL HEALTH (Part 3)

When assessing the long term effects of antiplaque agents, clinical trials have ranged from four weeks (Baab and Johnson 1989; Kozlovsky et al. 1994; Schaeken et al. 1994; Hase et al. 1995) to 3 months (De La Rosa and Sturzenberger 1976; Saxer et al. 1995; Binney et al. 1996; Eaton et al. 1997) to 6 months (Flötra et al. 1972; Baab and Johnson 1989; Kozlovsky et al. 1994; Schaeken et al. 1994; Hase et al. 1995). A clinical trial of four weeks does not fully allow significant long term effects of agents to be evaluated. Hence, small changes in staining or plaque growth may not be highlighted in four weeks, as they may be over six months. In order to facilitate maximum compliance and to fit into a tight schedule, a 6 week clinical trial was designed. A trial

of this length allowed a relatively quick evaluation of a test preparation of its plaque inhibitory activity and stain effects.

3.2. PROTOCOL

Approval for the following studies was received from the Human Research Ethics Committee, University of Adelaide, approval number H/16/98 and H/16/98a for Part 1 of the study involving chlorhexidine containing toothpaste (Appendix XII); and approval number H/15/98 for Parts 2 and 3 involving TTO containing mouthwash (Appendix XIII). Approval from the South Australian Dental Service-ethics subcommittee was also received prior to commencement of trials in the Adelaide Dental Hospital. Part 1 of this study was supported by Hamilton Laboratories, Adelaide, South Australia; and Parts 2 and 3 were supported by the Australian Tea Tree Oil Research Institute, Southern Cross University, Lismore New South Wales. An application for retrospective approval for Parts 2 and 3 was submitted after the completion of this thesis.

CHLORHEXIDINE 4 DAY PLAQUE GROWTH (Part 1)

Subjects were included if they had a clear medical history (ie. not suffering from any systemic diseases such as diabetes, hepatitis, cardiovascular or respiratory disease), had at least 20 natural teeth and were non-smokers. The exclusion criteria were subjects with periodontal pockets greater than 4mm, any illnesses, were on medication, or were pregnant.

This study was a randomised, blind, crossover clinical trial, balanced for residual effects. The randomisation pattern was computer generated. Double blindness was ensured for the three toothpastes. The fourth formula was the chlorhexidine mouthwash, issued as a blue liquid. Single-blindness of the assessor was maintained as all the formulas were

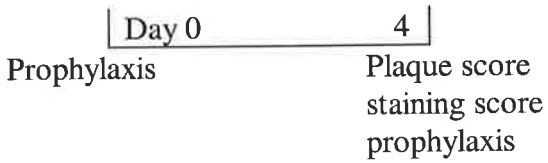
issued in identical coded white rectangular boxes. Taking this into consideration, the mouthwash preparation was considered as a single blind aspect of this study.

Measured lengths of toothpaste (2 grams) were placed in plastic vials by Hamilton Laboratories and delivered to the clinical trial investigator one week prior to the commencement of the trial. Due to the commercial sensitivity, the components of the test preparations were unknown. These preparations were not diluted into slurries prior to issue to the subjects, because the preservative would have been diluted and as some preparations would have been stored at room temperature for a few months, bacterial growth may have been encouraged. The preparations were issued in vials, and the subjects were required to add 10ml of water, stir the mixture for 30 seconds, and shake the vials for a minute to ensure maximum incorporation of the toothpaste into solution. Subjects were asked to rinse with 10ml of solution, for 60 seconds, twice a day. The chlorhexidine mouthwash was pre-measured and placed into identical vials and boxes as the toothpaste preparations.

Each subject underwent the same procedure 4 times (using a different preparation each time). Thirty healthy volunteers completed the study, 17 females and 13 males (18-44 years old) and were recruited from the tertiary institutions in South Australia. While fluctuations have been reported in gingival crevicular fluid flow at various stages of the menstruation cycle in females with pre-existing gingivitis (Holm-Pedersen and Löe 1967), hormonal variations were not considered to have had significant influence on the 4 day plaque growth study. In addition, the restricted time frame and limited resources prevented any consideration of the effects of different stages of the menstruation cycle in

the female subjects on the oral physiology. The scale and clean at day zero would have removed the plaque contributing to any gingivitis present.

Figure 3.1 Study outline:



At the first visit (Day 0), the subjects were given the information sheet (Appendix 1) to read and asked to sign the written consent form (Appendix II). These consent forms were witnessed and the nature of the clinical trial was explained to the subjects. The subjects received a dental examination and a scale and clean, followed by a dental prophylaxis to remove all plaque. Two photographs were taken, one of the extended tongue and the other of the labial surfaces of the teeth in an 'edge to edge' occlusion (with the cheeks retracted).

The subjects were issued with a coded container with one of the following formulations:

Table 3.1 Preparations tested

	Preparation
1	Chlorhexidine toothpaste slurry
2	Non chlorhexidine toothpaste slurry
3	Colgate Total® toothpaste slurry
4	0.12% chlorhexidine mouthwash

Subjects were requested not to use any mechanical form of oral hygiene during the 4 days of the study; specifically to refrain from brushing, flossing or using toothpicks. In addition, subjects were instructed not to chew gum. Chewing gum has been shown to

reduce occlusal plaque accumulation (Levinkind et al. 1999), and may alter the amount of plaque formed on the buccal and lingual surfaces.

On Day 4, when the subjects returned to the clinic, they were questioned on the taste acceptability of the preparations and were requested to rate the mouthrinse on a scale of 1 to 4, (1 being acceptable and 4 being unacceptable). Two photographs were taken, as described above. The teeth were scored for extrinsic staining and the subjects then rinsed with a plaque disclosing solution for one minute. After rinsing, the subjects were instructed to expectorate the excess solution and to rinse once gently with water. A third photograph was taken, this time of the plaque disclosed labial surfaces and a plaque score was recorded.

The Discolouration Index system used is described in Table 3.34 and the plaque scoring system used is in Table 3.3. Staining and plaque were removed with an ultrasonic scaler and a prophylaxis.

Following a 'wash out' period of at least 16 days to negate any carry-over effects of active ingredients in the mouthwashes, each subject returned to repeat the procedure with one of the other preparations. The schedule of appointments are in Appendix III. This process was repeated until all the preparations were tested. At the final appointment, each subject received a cash gratuity of $200.

TTO 4 DAY PLAQUE GROWTH (Part 2)

This was a randomised double-blind cross over study of 4 preparations. The double blindness of the study was ensured with identical coded containers of similarly coloured liquids. Each subject underwent the same procedure 4 times (using a different preparation each time). Thirty healthy volunteers began in this study and were recruited from the tertiary institutions in South Australia. Twenty five volunteers completed the four treatments, 16 females and 9 males (18-40 years old). There was no significant effects of the female to male ratio. The same protocol and inclusion criteria were used as described in Part 1 Section 1. Subjects were given an information sheet (Appendix IV) to read and asked to sign the written consent form (Appendix V). The nature of the clinical trial was explained to the subjects.

The subjects were issued with mouthwashes in coded bottles. They had to dispense 10ml into a pre-marked cup. They were asked to rinse this 10ml of solution, twice daily, for 60 seconds by the clock. During this four day period, they were asked not to perform any mechanical oral hygiene or to chew gum. These preparations were stored in a dry and cool (20-25 degree Celcius) environment and its volatile agents would not likely be released. The schedule of appointments are in Appendix VI.

The study design was identical to Part 1. The subjects were issued with a coded container with one of the following preparations:

Table 3.2 Preparations tested

	Preparation
1	tea tree oil mouthwash
2	Mouthwash base rinse
3	0.12% chlorhexidine mouthwash
4	Listerine mouthwash

On the final appointment, each subject received a cash gratuity of $200.

TEA TREE OIL 6 WEEK EFFECTS ON ORAL HEALTH (Part 3)

Volunteers were screened to select subjects having at least 6 sites of Papillary Bleeding Index (PBI) >2 and/or Gingival Index (GI) >1. Subjects were excluded if they had periodontal pockets greater than 4mm, any illnesses, were on medication, or were pregnant or were smokers. A total of 143 subjects were screened, and 63 were selected. They met the inclusion criteria of at least 20 natural teeth, at least six sites with Papillary Bleeding Index (PBI) score of at least 2, and/or Gingival Index (GI) score of at least two. Only 53 subjects attended the first appointment to participate in the study. Subjects were given an information sheet to read (Appendix VII) and asked to sign the written consent form (Appendix VIII).

The schedule of appointments is shown in Appendix VI.

Figure 3.2 Study outline:

Day 0	week 3	Week 6
Plaque score	Plaque score	Plaque score
Gingivitis score	Gingivitis score	Gingivitis score
Stain score	Stain score	Stain score
New toothbrush	New toothbrush	Scale & Clean
New toothpaste	New toothpaste	Prophylaxis

At the first visit (Day 0) the subject's medical history was checked and extrinsic stain level and gingival health (PBI & GI were scored. Two photographs were taken, one of the extended tongue and the second of the labial surfaces of the teeth in 'edge to edge' occlusion (with cheek retracted). The subjects then rinsed with a disclosing solution for 1 minute followed by rinsing once with water. A third photograph of the disclosed labial surfaces of the teeth in 'edge to edge' occlusion (with cheek retracted) was taken.

The Papillary bleeding index (PBI) used is described in Table 2.22 and the Gingival index in Table 2.11.

Following the baseline examination, the subjects were categorised according to their plaque and gingivitis scores. The subjects were then distributed amongst the test and control groups so that each group had similar oral health characteristics.

Subjects were issued with one of the following preparations:

Table 3.3 Preparations used

Group	*Preparation*
1	TTO mouthwash
2	Mouthwash base (no active agents)

The subjects were issued a box with 5 bottles of mouthrinse (200ml in each bottle), a new Colgate (Government standard) toothbrush, 2 tubes of Colgate regular toothpaste (45gm each tube) of sodium fluoride and 0.76% sodium monofluorophosphate. Subjects were instructed to place toothpaste along the entire length of the head of the toothbrush (approximately 2 grams) and brush as they normally would. No instruction on toothbrushing technique was given. After they had completed brushing, the subjects

were instructed to pour the mouthrinse into a portion cup (marked at 10ml) and to rinse for 60 seconds. The subjects were asked not to rinse or drink for 30 minutes after. This process was repeated twice a day. The subjects refrained from brushing and rinsing 24 hours prior to their next review appointment.

On Week 3, subjects returned to the clinic to have plaque level, extrinsic stain level and gingivitis scored and photographs taken as described above. The subjects were issued with a new toothbrush, 2 more tubes of toothpaste, and a new marked portion cup. No diaries were used to check compliance.

The same records were taken on Week 6 after which, subjects were given a scale and clean a dental prophylaxis and a cash gratuity of $50.

3.3. MATERIALS

The camera used was a Canon 50QD body, with a AF 100mm F2.8 macro lens, a macro ringlite ML3 adaptor and Cokin 52mm adaptor ring. The film used was Kodak Professional E100S Ektachrome Color Reversal Film 135. The films were processed at the Institute of Medical and Veterinary Science (IMVS) Photographic and Imaging laboratory.

In recording the papillary bleeding and the gingival indices, pressure sensitive probes (Pro-Dentec - Batesville, Arkansas USA) were used. These probes have a 0.55mm diameter ball-shaped point. A uniform pressure of 20 grams was used when probing the mesial and distal aspect of the dental papillae. This uniform pressure is achieved when the lower flexible arm of the probe point touched the fixed upper arm of the probe handle.

The plaque disclosing solution used was Colgate Disclogel, a 1%w/v erythrosine solution. 10 drops of the solution were placed into a portion cup, and the subjects were asked to rinse with this solution for 60 seconds, to distribute the solution evenly throughout their mouths.

The dental prophylaxis paste used was Colgate Neutrafluor®, containing neutral sodium fluoride (1.2%w/w), pumice alumina abrasives, saccharin, methyl hydroxybenzoate, propyl hydroxybenzoate. This was applied on the teeth with a rubber cup on a slow speed handpiece.

CHAPTER 4

RESULTS

Statistical Analyses

The data were analysed by the SPLUS statistical package; analysis of variance tables have been used to identify significant effects. The results of the analyses have been reported to two decimal places to distinguish between relatively small differences. The statistical difference in all the analyses were determined by the value of the standard deviation of difference of mean values; this is otherwise known as the standard error of that difference (SDdif). For statistical significance at the 5% level between a pair of means, the difference must be greater than twice the SDdif.

Table 4.5 is used to illustrate this analysis.

Table 4.5

Statistically significant differences of mean plaque scores between the four preparations.

SDdif 0.063	chlorhexidine toothpaste	placebo	Colgate Total®	0.12% chlorhexidine mouthwash
ch toothpaste	-	0.28	not significant	0.65
placebo	-	-	0.22	0.93
Colgate Total®	-	-	-	0.71

There were significant differences between all combinations of the four preparations except between chlorhexidine toothpaste and Colgate Total® toothpaste. The figures in Table 4.5 are the differences between the mean plaque index scores of the four preparations. For example, the difference between the mean plaque score of chlorhexidine toothpaste (3.17) and placebo (3.46) was 0.28 (with rounding error). The SDdif for this comparison was 0.063. Therefore, there was a statistically significant

difference between the chlorhexidine toothpaste and placebo because 0.28 is greater than twice the SDdif of 0.126.

The same pattern of data reporting is used consistently throughout the tables.

Part 1 Chlorhexidine and Part 2 TTO 4 day plaque growth clinical studies

Two variables (plaque and stain indices) were analysed in relation to the four preparations tested in each of the Parts 1 and 2. Third molars were excluded from the study. Subjects were assigned a maximum of 56 scores for each index per visit (ie. two surfaces of 28 teeth). If the subject has less than 28 natural teeth, the mean score was obtained according to the number of teeth scored.

For subjects with 28 teeth, the following calculations were made for the three different types of analyses. In the first analysis, these 56 scores (28 teeth buccal and lingual surfaces) were added and then divided by 56 to give the **mean score** for that index.

The second analysis involved allocating data (ie. all 56 values) per index per person, into 12 values to correspond to the 12 positions in the mouth (ie. buccal and lingual surfaces of maxillary and mandibular teeth in anterior and posterior teeth groups). This analysis further took into account the effects of the interaction of the position in the mouth on preparations, using FDI notation (Table 4.1).

Table 4.1

Distribution of teeth and surfaces into the 12 values for the analysis of 28 teeth

Maxilla

buccal	17-14	13-23	24-27
lingual	17-14	13-23	24-27

Mandible

buccal	47-44	43-33	34-37
lingual	47-44	43-33	34-37

(FDI tooth notation)

The third analysis is similar to the second except that only the 20 non molar teeth were included in the 12 positions. The teeth were divided into: incisors; and canine and premolars (Table 4.2).

Table 4.2

Distribution of teeth and surfaces into the 12 values for the analysis of 20 teeth

Maxilla

buccal	15-13	12-22	23-25
lingual	15-13	12-22	23-25

Mandible

buccal	45-43	42-32	33-35
lingual	45-43	42-32	33-35

Part 3 TTO 6 week effects on oral health

The data were analysed by the SPLUS statistical package. There were four variables (plaque, stain, gingival and bleeding indices) which were analysed in relation to the two preparations. The first analysis involved comparison of mean values at three times (weeks zero, three and six) within each variable/index. The second and third analysis were identical to those described for Parts 1 and 2.

Subject Demographics

Table 4.3 represents details of subject numbers, age and sex.

Table 4.3

Summary of subject demographics

	Female	Male	25 years or less	over 25 years	Total (n)
Part 1 chlorhexidine 4 day - randomised	17	13	23	7	30
Part 2 TTO 4 day - randomised	16	9	18	7	25
Part 3 TTO 6 weeks	24	25	42	7	49

4.1 CHLORHEXIDINE 4 DAY PLAQUE GROWTH (PART 1)

Chlorhexidine toothpaste

Due to the variations in calculations, the plaque index score between mean, 28 teeth and 20 teeth analyses were not identical, but similar (Table 4.4).

Table 4.4

Plaque index scores for mean score, analysis with 28 teeth and analysis with 20 teeth.

Number of teeth	chlorhexidine toothpaste	placebo	Colgate Total ®	0.12% chlorhexidine mouthwash
mean score (SDdif 0.063)	3.17	3.46	3.23	2.53
28 teeth (SDdif 0.065)	3.19	3.50	3.26	2.45
20 teeth (SDdif 0.072)	3.11	3.44	3.14	2.43

However, the order (or ranking) from the lowest to highest plaque index score was the same in all three analyses:

1. chlorhexidine mouthwash was the lowest,
2. chlorhexidine toothpaste,
3. Colgate Total® and
4. placebo.

The ranking of plaque index score is best represented by photographs of a high plaque former at day 4 after the use of each preparation (Figure 4.2). The same preparations had less of an impact on a low plaque former (Figure 4.3).

With 28 teeth, the analysis of variance showed large differences between subjects ($p<0.001$) and very large differences between the four preparations ($p<0.001$). With 20

teeth, there were large differences between preparations ($p<0.001$), and the findings were in strong agreement with the analysis with 28 teeth.

Plaque index

There were significant differences between all comparisons of the four preparations except between chlorhexidine toothpaste and Colgate Total® toothpaste (Table 4.5).

Table 4.5

Statistically significant differences of mean plaque scores between the four preparations.

SDdif 0.063	chlorhexidine toothpaste	placebo	Colgate Total®	0.12% chlorhexidine mouthwash
chlorhexidine toothpaste	-	0.28	not significant	0.65
placebo	-	-	0.22	0.93
Colgate Total®	-	-	-	0.71

Plaque index - analysis with 28 teeth

There were large differences between preparations ($p<0.001$) and significant interaction with time. In all preparations the buccal surfaces had the higher plaque score when compared with the lingual surfaces. In all preparations (except for Colgate Total®), the mandibular teeth had the higher plaque score when compared with the maxillary teeth.

There was interaction between preparation and position which changed with time. When considering different positions in the mouth (ie. anterior or posterior), the ranking of plaque index scores from the lowest to the highest changes depending on the time during the clinical trial (Table 4.6).

Table 4.6

Ranking of preparations from the lowest to the highest plaque index score of the different teeth positions, over the four times.

Teeth (FDI notation)	time	0.12% chlorhexidine mouthwash	chlorhexidine toothpaste	Colgate Total®	placebo
17-14, 47-44	1	1	2	4	3
	2	1	3	2	4
	3	1	2	3	4
	4	1	2	4	3
13-23, 43-33	1	1	3	2	4
	2	1	3	2	4
	3	1	2	3	4
	4	1	2	3	4
24-27, 34-37	31	1	2	3	4
	2	1	3	2	4
	3	1	2	3	4
	4	1	2	3	4

The 0.12% chlorhexidine mouthwash was the most consistent in its ranking as resulting in the lowest plaque index score, and the placebo with the highest plaque index score. The chlorhexidine toothpaste had the second lowest plaque score on twice as many occasions when compared with the Colgate Total®. However, the mean plaque scores between the chlorhexidine toothpaste and Colgate Total® were not significantly different. The chlorhexidine toothpaste plaque index score was lower in the posterior teeth when compared to Colgate Total®.

Comparison of analyses between 28 and 20 teeth for plaque index

There was strong agreement between the analyses for 28 teeth and 20 teeth. However, there was an increase in residual variance, and mainly decreases in F values (Appendix IX).

Stain index

There were strong differences due to preparations (*p=0.0003*) in the analysis of mean stain scores (Table 4.7). Due to the variations in calculations, the stain index scores were not identical, but were similar.

Table 4.7

Stain index scores for total mean score, analysis with 28 teeth and 20 teeth.

Number of teeth	chlorhexidine toothpaste	placebo	Colgate Total®	0.12% chlorhexidine mouthwash
total mean score (SDdif 0.059)	0.17	0.16	0.11	0.36
28 teeth (SDdif 0.047)	0.14	0.14	0.09	0.32
20 teeth (SDdif 0.07)	0.22	0.21	0.15	0.44

The order from the highest to lowest stain index score was the same in all three analyses, with

1. chlorhexidine mouthwash was the highest,
2. chlorhexidine toothpaste,
3. placebo and
4. Colgate Total®

There were significant differences (SDdif 0.059) in mean stain index scores between the positive control 0.12%chlorhexidine mouthwash and all the other three preparations. No other comparisons were significantly different (Table 4.8).

Table 4.8

Analysis of mean stain scores between the four preparations.

SDdif 0.059	chlorhexidine toothpaste	placebo	Colgate Total®	0.12% chlorhexidine mouthwash
chlorhexidine toothpaste	-	not significant	not significant	0.19
placebo	-	-	not significant	0.20
Colgate Total®	-	-	-	0.25

Stain index - analysis with 28 teeth

There were strong differences due to preparations (*p=0.0004*), and these did not interact with time. The interaction between preparation and jaw changed with time. When considering the maxilla and mandible, the ranking of stain index scores from the highest to lowest changed depending on the time during the clinical trial (Table 4.9).

Table 4.9

Ranking of preparations from the highest to lowest maxillary and mandibular stain index scores over the four times.

Teeth	time	0.12% chlorhexidine mouthwash	chlorhexidine toothpaste	Colgate Total®	placebo
Maxilla	1	1	3	2	4
	2	1	2	2	2
	3	2	2	2	1
	4	1	4	3	2
Mandible	1	1	3	2	4
	2	1	2	3	4
	3	1	3	4	2
	4	1	4	3	2

Comparison of analysis between 28 and 20 teeth for stain index

There was strong agreement between the analysis for 28 teeth and 20 teeth. However, there was an increase in residual variance, and mainly decreases in F values (Appendix IX).

Taste rating

There were strong differences between preparations (*p=0.001*); and between subjects (*p=0.02*); with regard to taste (Table 4.10). The most unacceptable preparation was

1. chlorhexidine mouthwash,
2. followed by chlorhexidine toothpaste and Colgate Total®, and
3. the placebo was the most acceptable
4.

Table 4.10

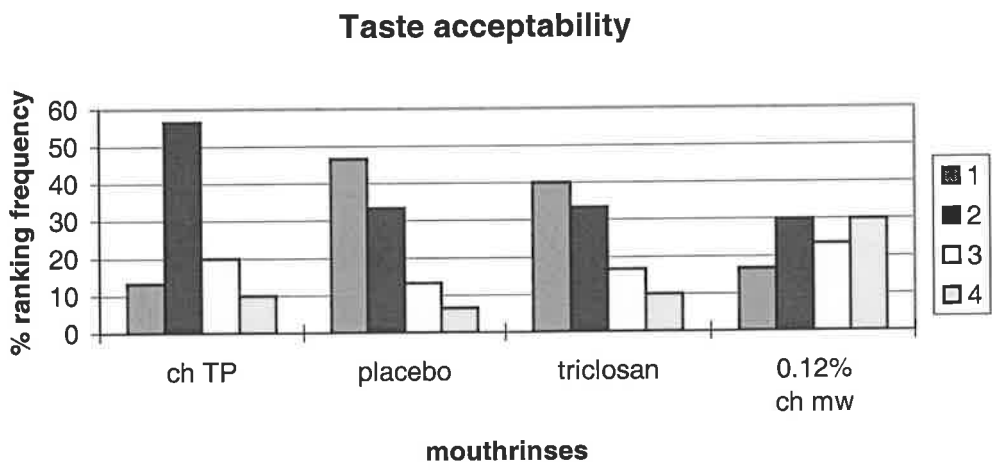
Mean taste scores for four preparations.

Chlorhexidine toothpaste	placebo	Colgate Total®	0.12% chlorhexidine mouthwash	SDdif 0.222
2.27	1.80	1.97	2.67	

The frequency of ranking of taste is shown in Figure 4.3.

Figure 4.1

Ranking frequencies of taste in percentage



There were significant differences between the taste scores of 0.12% chlorhexidine mouthwash and all the other three preparations. In addition, there was a significant difference between chlorhexidine toothpaste and the placebo (Table 4.11).

Table 4.11

Analysis of mean taste scores between the four preparations.

SDdif 0.222	chlorhexidine toothpaste	placebo	Colgate Total®	0.12% chlorhexidine mouthwash
chlorhexidine toothpaste	-	0.55	not significant	0.40
placebo	-	-	not significant	0.87
Colgate® Total	-	-	-	0.70

Taste rating

Table 4.12 represents the ranking of taste scores.

Table 4.12

Mean taste scores are:

chlorhexidine toothpaste	placebo	Colgate Total®	0.12% chlorhexidine mouthwash
2.8	1.8	1.4	3.6
SDdif 0.33			

The most to least acceptable taste preparations in terms of were :

1. Colgate Total®,
2. placebo,
3. chlorhexidine toothpaste, and
4. 0.12% chlorhexidine mouthwash.

Figure 4.2

High plaque former (subject number 2020)

Photograph of labial surfaces at Day 4 after the use of:

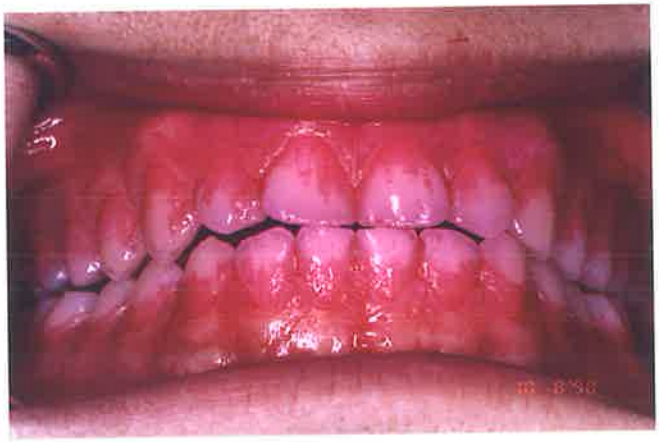
A. chlorhexidine toothpaste

B. placebo

C. Colgate Total ®

D. 0.12% chlorhexidine mouthwash

A



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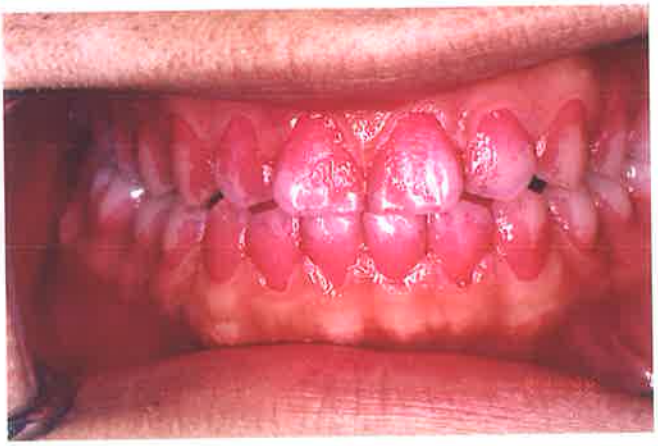

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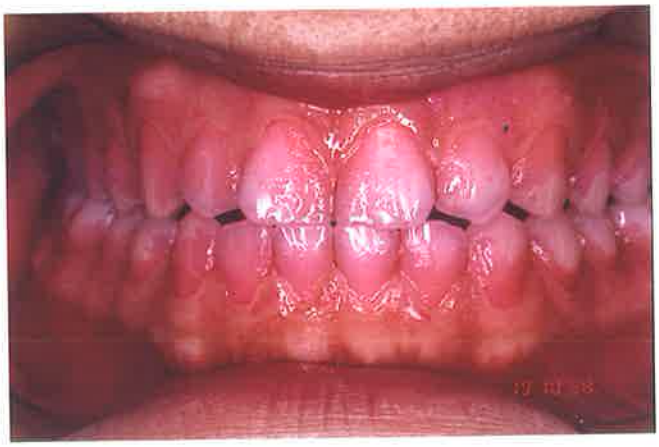

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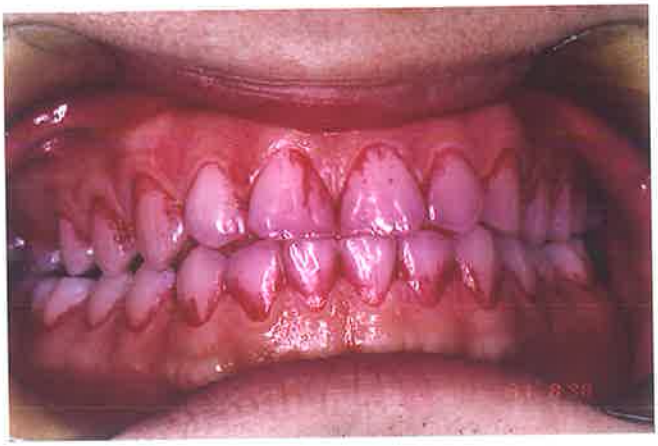


Figure 4.3

Low plaque former (subject number 2005)

Photograph of labial surfaces at Day 4 after the use of:

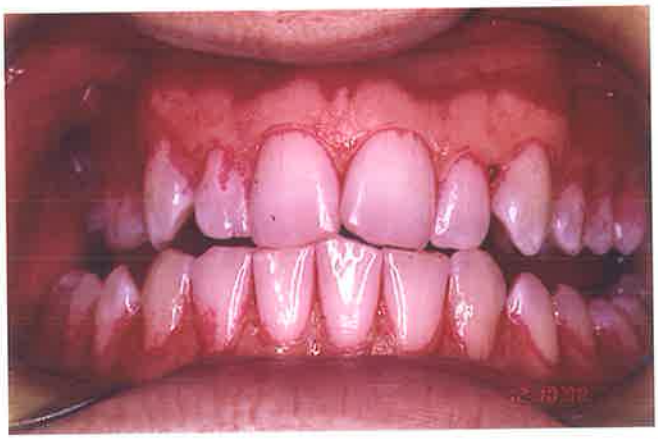
A. chlorhexidine toothpaste

B. placebo

C. Colgate Total®

D. 0.12% chlorhexidine mouthwash

A



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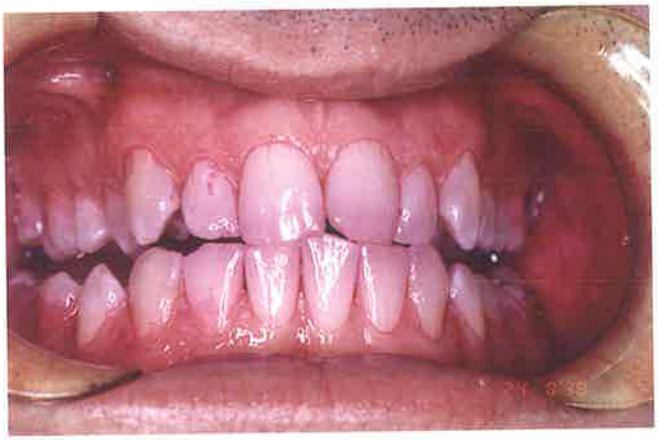

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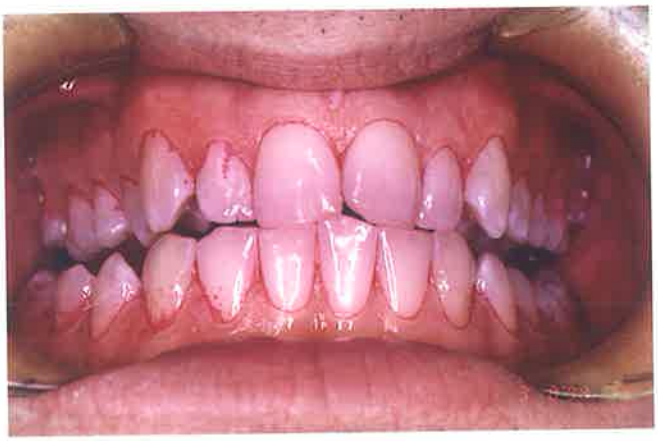

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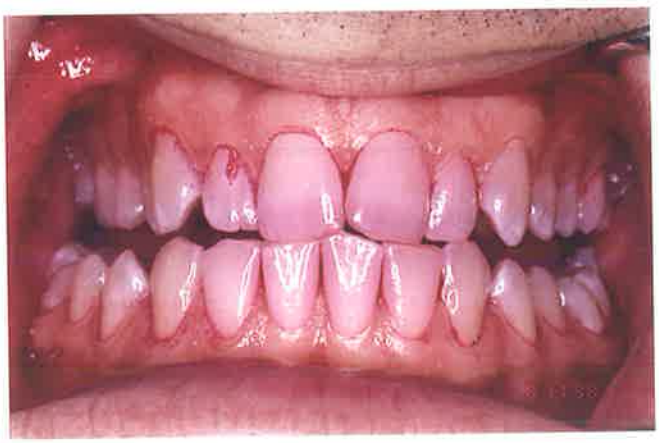


4.2 TTO 4 DAY PLAQUE GROWTH (PART 2)

Plaque index

The preparations were not evenly balanced between times resulting in an apparent effect from interaction of preparation and time. There was a strong effect of preparation ($p<0.001$) (Table 4.13).

Table 4.13

Plaque index scores for mean score and analyses of 28 teeth.

number of teeth	TTO	placebo	0.12% chlorhexidine mouthwash	Listerine®
total mean score (SDdif 0.081)	3.04	3.59	3.34	3.13
28 teeth (SDdif 0.076)	3.04	3.56	3.32	3.13
20 teeth (SDdif 0.094)	2.99	3.60	3.28	3.02

The TTO mouthwash resulted in the lowest plaque score, the second lowest was Listerine® followed by 0.12% chlorhexidine mouthwash and the placebo. This data indicated that the positive control 0.12% chlorhexidine mouthwash was supplied to the examiner in an inactive state as its plaque inhibitory effect was only marginally better than the placebo. The ranking of plaque index score is best represented by photographs of a high plaque former at day 4 after the use of each preparation (Figure 4.4).

There were significant differences between all preparations, with the exception of TTO mouthwash and Listerine®. There were no significant differences between the 4 times at which the measurements were made ($p=0.142$), nor were the interactions between preparation and time significant (Table 4.14).

Table 4.14

Analysis of mean plaque scores (four preparations).

SDdif 0.222	TTO	placebo	0.12% chlorhexidine mouthwash	Listerine®
TTO	-	0.54	0.29	not significant
placebo	-	-	0.25	0.46
0.12% chlorhexidine mouthwash	-	-	-	0.21
Listerine®	-	-	-	-

The analyses with 28 teeth were in strong agreement with the mean plaque index score analysis. The overall ranking of plaque score from the lowest to the highest was:

1. TTO,
2. Listerine®,
3. chlorhexidine and
4. placebo.

In the preparation and surface interactions, this ranking (from lowest to highest plaque score) changed on buccal and lingual surfaces over time. (Table 4.15)

Table 4.15

Ranking of preparations plaque index score (lowest to highest).

Teeth	time	Listerine®	TTO	0.12% chlorhexidine mouthwash	placebo
Buccal	1	1	2	3	4
	2	2	1	3	4
	3	1	4	3	2
	4	2	1	3	4
Lingual	1	2	1	4	3
	2	3	1	2	4
	3	2	4	1	3
	4	2	1	3	4

The placebo preparation was the only preparation which was consistent in its ranking. TTO mouthwash showed the widest variation, and was ranked the lowest most of the time and the highest on several occasions.

Comparison of analysis between 28 and 20 teeth for plaque index

There was strong agreement between the analysis for 28 teeth and 20 teeth. However, there was an increase in residual variance, and mainly decreases in F values (Appendix X).

Stain index

There were no effects of preparation on the mean stain score ($p=0.238$) (Table 4.16).

Table 4.16

Stain index scores for mean score, analyses with 28 and 20 teeth.

Number of teeth	TTO	placebo	0.12% chlorhexidine mouthwash	Listerine®
total mean score (SDdif 0.057)	0.20	0.08	0.19	0.16
28 teeth (SDdif 0.05)	0.18	0.06	0.16	0.13
20 teeth (SDdif 0.072)	0.24	0.09	0.25	0.21

There was interaction between preparation and jaw and the way this changed over time. Within the maxilla at time 1, there was a significant difference between the Listerine® and placebo, after which the stain score in the maxilla became non existent. Regardless of the preparation used, the stain score in the maxilla decreased dramatically after time 1 by a factor of ten.

Comparison of analysis between 28 and 20 teeth for stain index

There was strong agreement between the analysis for 28 teeth and 20 teeth. However, there was an increase in residual variance, and mainly decreases in F values (Appendix XI).

Taste rating

There were differences between preparations, and no evidence of differences between subjects or over time (Table 4.17).

Table 4.17

Mean taste scores for four preparations.

TTO	placebo	0.12% chlorhexidine mouthwash	Listerine®	SDdif 0.22
2.6	1.72	1.76	3.16	

The most unacceptable preparation was Listerine®, followed by the TTO mouthwash. The placebo and 0.12% chlorhexidine mouthwash were the most acceptable.

Figure 4.4

High plaque former (subject number 115)

Photograph of labial surfaces at Day 4 after the use of:

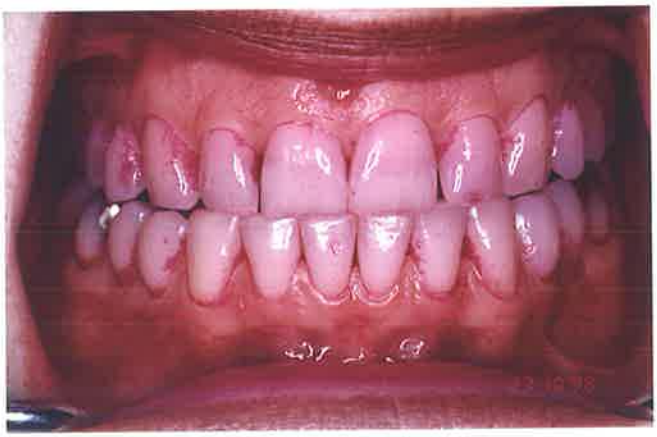
A. TTO mouthwash version 1

B. placebo

C. 0.12% chlorhexidine mouthwash

D. Listerine®

A



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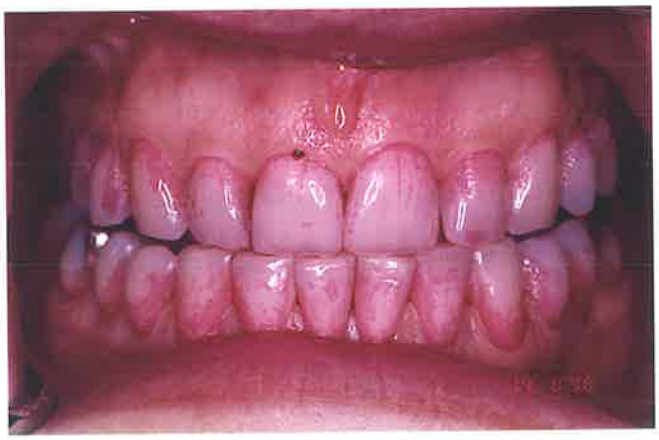

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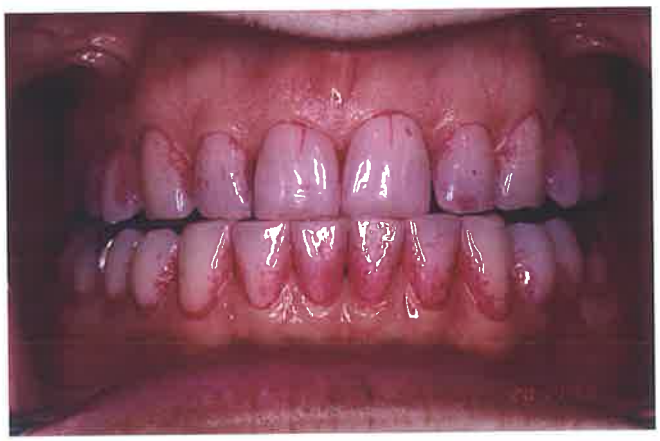

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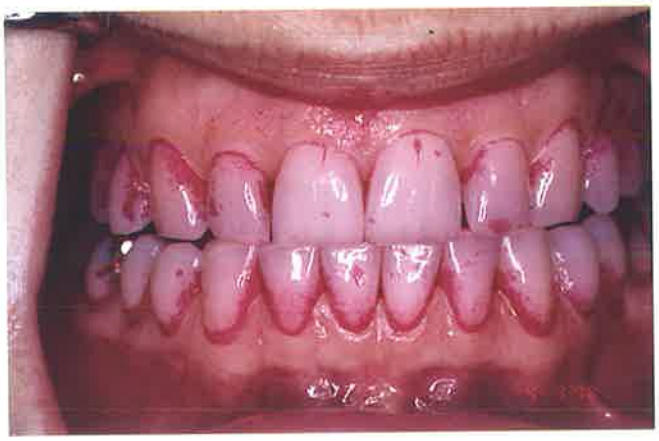


4.3 TTO 6 WEEK EFFECTS ON ORAL HEALTH

Plaque Index

The analysis of variance (ANOVA) tested for preparations against mean plaque scores (Table 4.18) are graphically represented in Figure 4.5.

Table 4.18

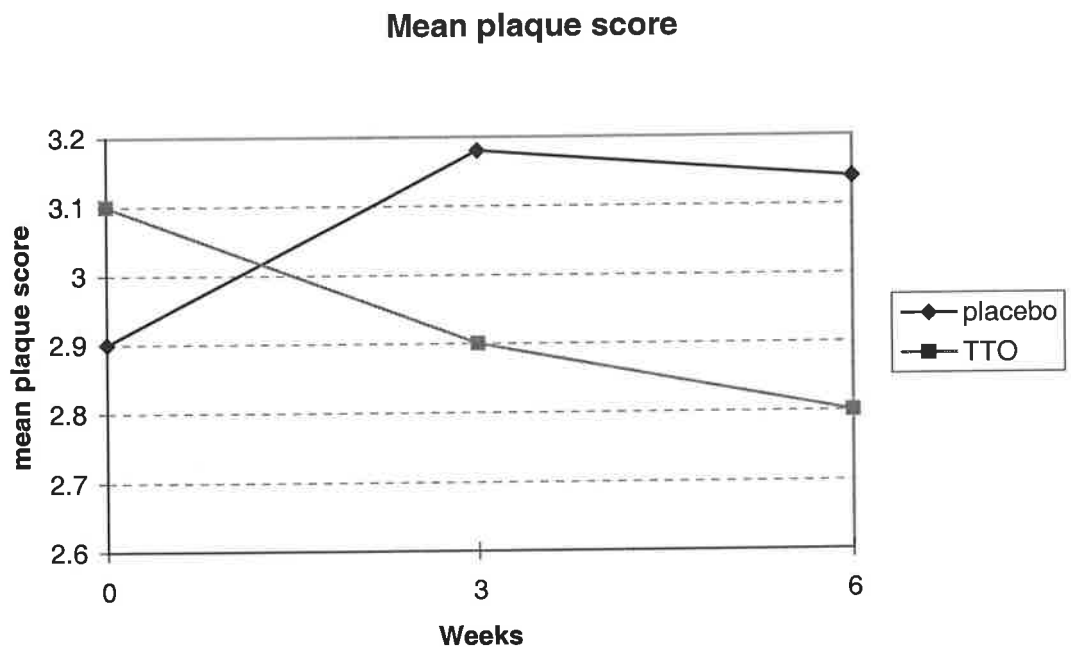
Mean plaque index scores at weeks 0, 3 and 6

	week 0	week 3	week 6	SDdif = 0.085
TTO version 1	3.09	2.9	2.81	2.93
placebo	2.91	3.18	3.14	3.07
SDdif = 0.093	3.00	3.03	2.96	3.00

At week 0, there was no significant difference between the two preparations; at week 3, there was a significant difference between the two preparations and at week 6, there was an even greater significant difference of 0.33. In the TTO group, the plaque score decreased by about 6% at week 3, and decreased by 9% at week 6 in relation to the baseline plaque score. In the placebo group, the plaque score increased by about 9% and 8% at week 3 and 6 respectively, in relation to the baseline score.

Figure 4.5

Mean plaque index scores at weeks 0, 3 and 6



There was a strong interaction between time and preparations ($p<0.001$). The plaque score for the TTO preparation decreased with time and the score for the placebo preparations increased with time. Within the TTO preparation, there were significant differences between weeks 0 and 3 (with a difference of 0.19); and between week 0 and 6 (with a difference of 0.28); with the overall effect of decreasing plaque scores. Within the placebo preparation, there were significant differences between weeks 0 and 3 (with a difference of 0.27) and weeks 0 and 6 (with a difference of 0.23); with an overall increase in plaque scores.

Week 0 to 3

The analysis involving 28 teeth for week 0 to 3 period revealed large effects due to preparations ($p=0.003$) and surface ($p<0.001$). The plaque score increased on the buccal and decreased on lingual surfaces. The overall plaque index score increased for

the placebo (0.28) and decreased for TTO preparation. There were significant differences between the plaque index scores for TTO that of the placebo. There were significant differences within the buccal and lingual surfaces, and within each preparation (Table 4.19).

Table 4.19

Mean plaque index scores for buccal and lingual surfaces at weeks 0 to 3

	Buccal	Lingual	SDdif = 0.056
TTO	0.02	-0.38	-0.18
placebo	0.46	0.10	0.28
SDdif = 0.147	0.233	-0.147	0.043

Week 0 to 6

There was a strong effects of preparations *(p=1.75e-05).* (Table 4.20)

Table 4.20

Plaque index scores for mean score, analysis with 28 and 20 teeth at weeks 0 to 6.

Number of teeth	TTO mouthwash	placebo
mean plaque index score	-0.28	0.23
28 teeth (SDdif 0.107)	-0.27	0.24
20 teeth (SDdif 0.117)	-0.31	0.23

Week 3 to 6

Table 4.21 represents that there was a weak interaction between preparation and surfaces of teeth *(p=0.001).*

Table 4.21

Mean plaque index scores for buccal and lingual surfaces at weeks 3 to 6

	Buccal	Lingual	SDdif = 0.070
TTO	-0.15	-0.01	
placebo	0.03	-0.13	
SDdif = 0.134			

Between weeks 3 to 6, within the placebo preparation, there was a significant difference (SDdif 0.070) between buccal and lingual surfaces (with a difference of 0.16). No other comparisons were statistically significant. Both preparations showed a similar overall decrease in plaque score over this period. However, the placebo preparation showed an increase in the buccal plaque score (0.029), with a relatively large decrease in the lingual plaque score (-0.128). The TTO preparation had a decrease in plaque score on both surfaces, but the decrease on the lingual surface was relatively small (-0.007).

Comparison of 28 and 20 teeth analysis for plaque index

There was strong agreement between the analyses of 28 and 20 teeth. There was an increase in residual variance and mainly decreases in F values in the 20 teeth analysis, in comparison to the 28 teeth analysis (Appendix XI). The plaque index scores the period week 0 to 3 are shown in Table 4.22.

Table 4.22

Plaque index scores for mean score, analysis with 28 and 20 teeth at weeks 0 to 3.

Number of teeth	TTO mouthwash	placebo
mean plaque index score	-0.19	0.27
28 teeth (SDdif 0.147)	-0.02	0.28
20 teeth (SDdif 0.161)	-0.24	0.28

There were strong effects of preparations *($p=0.002$)* and some effect of surface *($p<0.001$)*. The overall plaque score decreased for TTO preparation (-0.24) and increased for the placebo preparation (0.28) between weeks 0 and 3. With the TTO preparation, both tooth surfaces showed a decrease in plaque score (buccal -0.15, lingual -0.01). The analysis of 20 teeth highlighted a small increase in buccal surface scores. This is in contrast to the 28 teeth analysis, where a small decrease in buccal surface scores is seen.

Stain Index

The ANOVA tested for effects of preparations against subjects' mean stain scores. Analysis of the mean stain index score revealed that there was no effect of preparations between the two groups of subjects. There were strong differences between times *(p=0.001)* and a strong interaction between preparation and time *(p=0.002)* (Table 4.23).

Table 4.23

Mean stain index scores

mean	week 0	week 3	week 6	SDdif = 0.059
TTO	0.24	0.39	0.55	0.39
placebo	0.25	0.21	0.25	0.23
SDdif = 0.101	0.24	0.29	0.40	

At baseline (week 0) there was no significant difference in stain scores between the subjects using TTO (0.24) and placebo (0.25) preparations. At week 6 there was a significant difference in stain scores between the subjects using TTO (0.55) and placebo (0.25) preparations. When the stain scores of both preparations were added together, there was a significant difference (SDdif 0.059) in stain scores between weeks 0 and 6 (with a difference of 0.15).

There were significant differences during the different time periods within the stain index scores for TTO mouthwash group (Table 4.24).

Table 4.24

The changes in mean stain index scores for TTO

mean	week 3-0	week 6-0	week 6-3	
TTO	0.15 sig	0.21 sig	0.16 sig	SDdif = 0.059

Within the TTO preparation, there were significant differences in stain scores between weeks 0 and 3, 0 and 6, and week 3 and 6.

The changes in mean stain index scores for both preparations over the three time periods (Table 4.25) are highlighted in Figure 4.6.

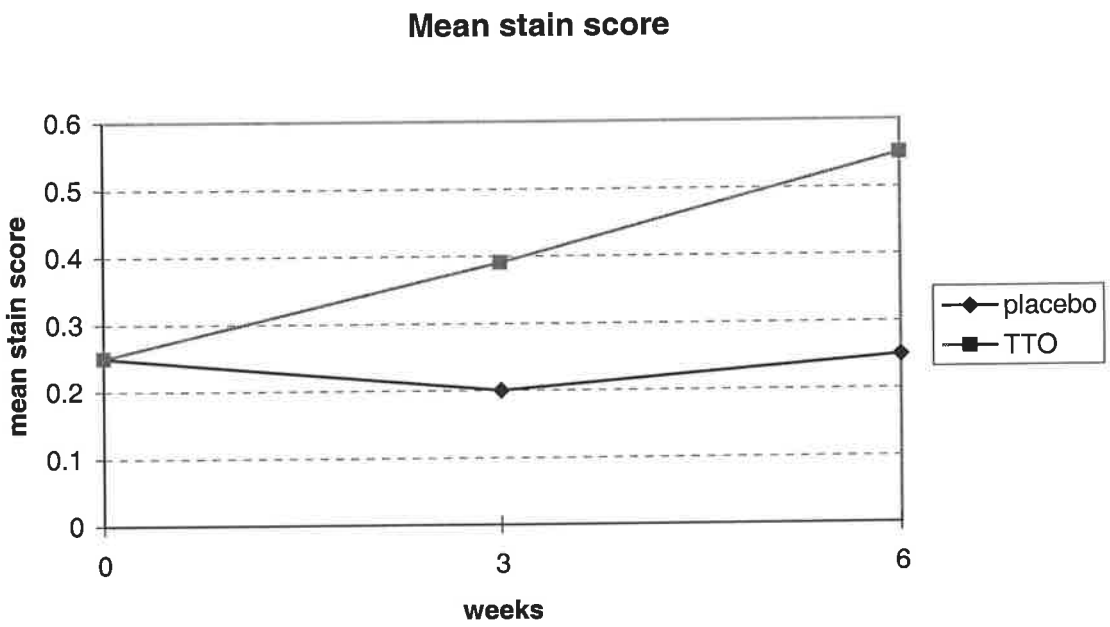
Table 4.25

The changes in stain index scores

mean	week 3-0	week 6-0	week 6-3
TTO	0.13	0.29	0.20
placebo	-0.04	-0.00	0.06
SDdif =	0.069	0.087	0.074

Figure 4.6

Mean stain index scores



With the analysis of 28 teeth, there were weak effects of preparation *(p=0.02)* over the week 0 to 3 period; strong effects of preparation *(p=0.0017)* over the week 0 to 6 period and no effects of preparations between weeks 3 and 6. There were significant differences between the stain scores over the week 0 to 3, and 0 to 6 periods for TTO and placebo preparations. Both preparations showed an increase in stain scores from

week 3 to 6, with the TTO preparation showing a greater increase (0.20 compared with 0.06).

Comparison of 28 and 20 teeth analysis for stain index

There was strong agreement between the analyses of 28 teeth and 20 teeth. The stain index scores with the 28 teeth analysis is shown in Table 4.26.

Table 4.26

The changes in stain index scores

28 teeth analysis	week 3-0	week 6-0	week 6-3
TTO	0.18	0.37	0.2
placebo	-0.04	0.02	0.06
SDdif =	0.106	0.114	0.087

With the analysis of 20 teeth during the week 0 to 3 period, there were strong effects of preparation *(p=0.04)*. There were significant differences between the TTO and placebo preparations. With the analysis of 20 teeth during the week 0 to 6 period, there were strong effects of preparation *(p=0.003)*. There were significant differences in changes in stain score between the TTO and placebo preparations.

With the analysis of 20 teeth during the week 3 to 6 period, there were no effects of preparation.

Gingival index

The analysis of mean gingival scores showed no effects of preparation, overall or over time. There were significant changes over time *(p=0.034)* for both preparations (Table 4.27), and are highlighted in Figure 4.7.

Table 4.27

Mean gingival index score

mean	week 0	week 3	week 6	SDdif = 0.053
TTO	0.59	0.50	0.46	
placebo	0.52	0.47	0.47	

There was a significant difference (SDdif 0.053) in the gingival score for 0 to 6 weeks for TTO preparation (with a difference of 0.13). With the analysis of 28 teeth, there were no effects of preparation on gingival index score at any time period (Table 4.28), and are highlighted at Figure 4.7.

Table 4.28

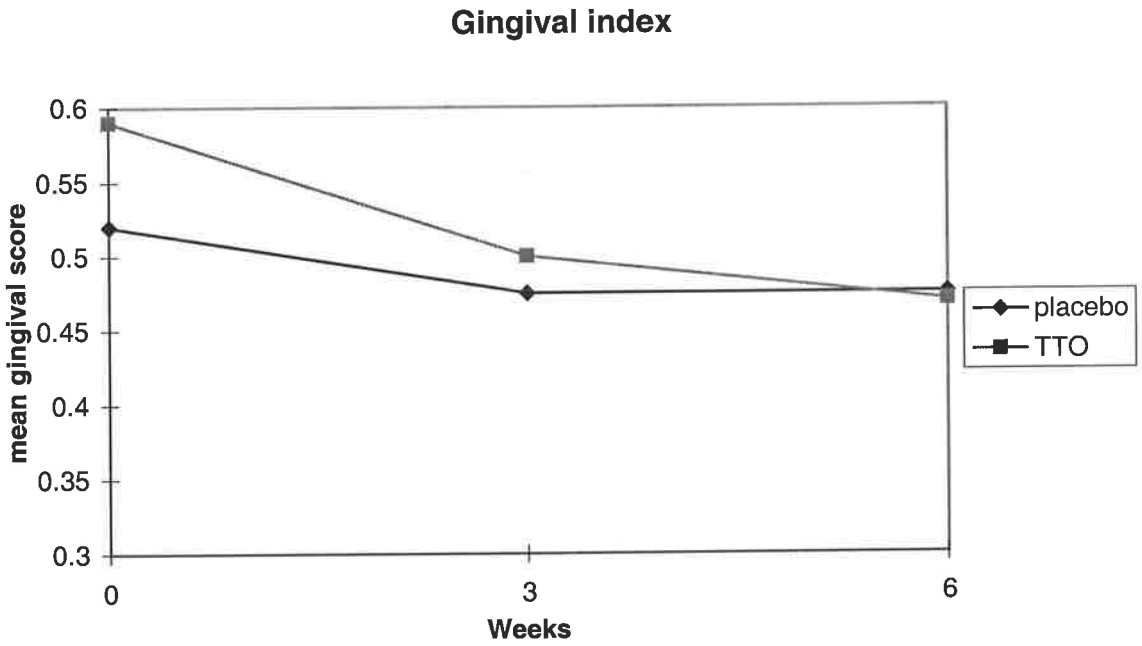
Gingival index scores over different time periods

28 teeth analysis	week 3-0	week 6-0	week 6-3
TTO	-0.09	0.014	0.01
placebo	-0.06	-0.05	0.04
SDdif =	0.083	0.078	0.076

There was no significant difference between the two preparations at the three points in time. In the TTO group, the gingival score decreased by 15% at week 3 and decreased by 22% at week 6, in relation to the baseline gingival score. In the placebo group, the gingival score decreased by 10% at week 3 and 6, in relation to the baseline gingival score.

Figure 4.7

Mean gingival index score



Comparison of 28 and 20 teeth analysis for gingival index

There was strong agreement between the analyses of 28 teeth and 20 teeth. With the analysis of 20 teeth, there were no effects of preparation over any of the time periods. (Table 4.29)

Table 4.29

Gingival index scores over different time periods

20 teeth analysis	week 3-0	week 6-0	week 6-3
TTO	-0.13	-0.11	0.01
placebo	-0.07	00.04	0.04
SDdif =	0.079	0.071	0.076

Bleeding index

The analysis of mean bleeding scores showed no effects of preparation, overall or over time. The majority of the variation consisted of the large differences between subjects. With the analysis of 28 teeth, there was no effect of preparation over any time period (Table 4.30), and are highlighted in Figure 4.8.

Table 4.30

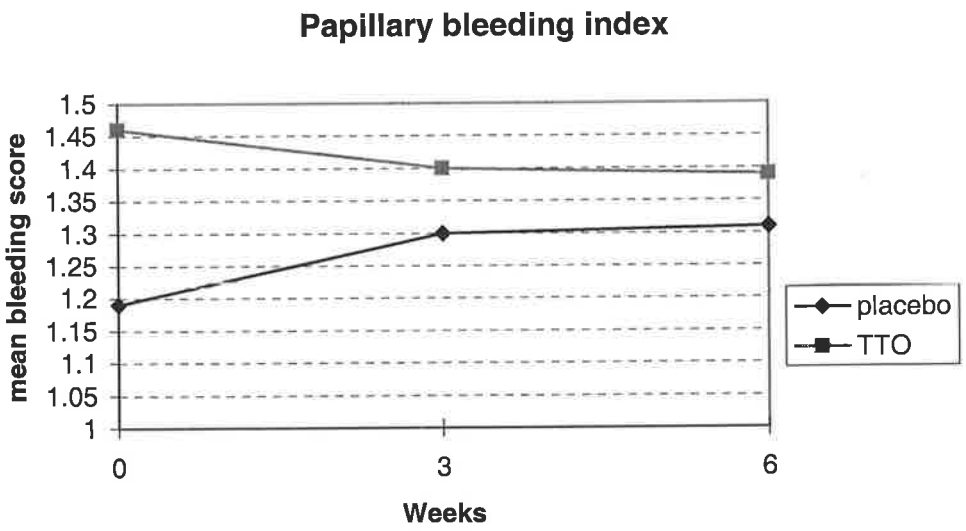
Bleeding index scores over different time periods

28 teeth analysis	week 3-0	week 6-0	week 6-3
TTO	-0.07	-0.11	-0.08
placebo	0.012	0.16	-0.05
SDdif =	0.160	0.147	0.165

There was no significant difference between the two groups at any of the three points in time.

Figure 4.8

Bleeding index score



Comparison of 28 and 20 teeth analyses for bleeding index

There was strong agreement between the analysis of 20 teeth and 28 teeth. There were no effects of preparation over any of the time periods (Table 4.31).

Table 4.31

Bleeding index scores over different time periods

20 teeth analysis	week 3-0	week 6-0	week 6-3
TTO	-0.05	-0.07	-0.08
placebo	0.15	0.06	-0.02
SDdif =	0.158	0.150	0.076

In the TTO group, the mean score decreased by about 15% and 22% at week 3 and 6 respectively from the baseline score. In the placebo group, the mean score decreased by about 10% at both week 3 and 6 from the baseline score.

Figure 4.9

Yellow film (subject number 155) after the use of TTO mouthwash

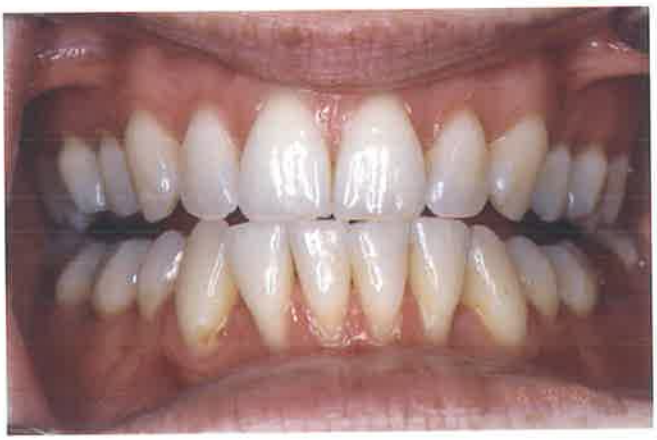
Photograph of labial surfaces at:

0 Week 0 (baseline)

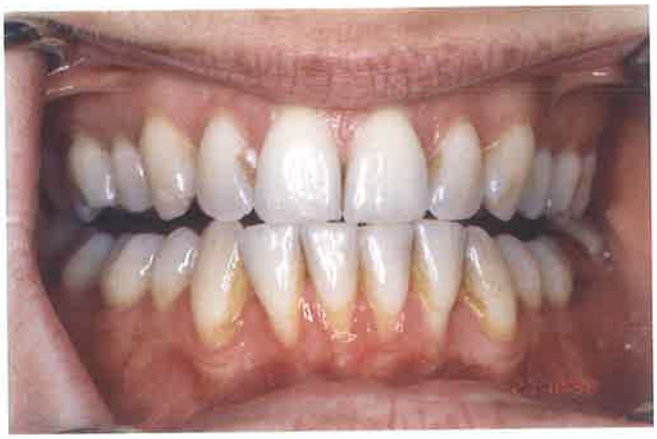
3 Week 3 (increased discolouration and yellow film)

6 Week 6 (marked staining and yellow film)

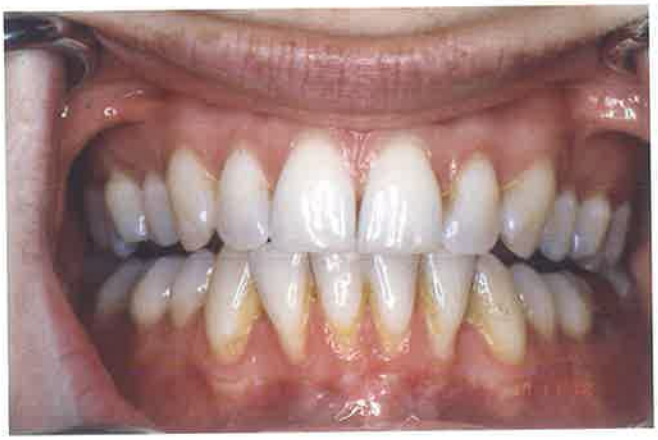
0



3



6



The following tables illustrates the consistency of this ranking for plaque and stain for each data set.

Table 4.32 Comparison of ranking of preparations using different data sets.

	Chx plaque	TTO plaque	Chx stain	TTO stain
mean score 28 teeth	√	√	√	√
maxillary teeth score (in 28 teeth analysis)	√	√	X	X
mandibular teeth score (in 28 teeth analysis)	√	√	√	√
buccal surface score (in 28 teeth analysis)	X	√	√	X
lingual surface score (in 28 teeth analysis)	√	√	X	√
mean score 20 teeth	√	√	√	√
maxillary teeth score (in 20 teeth analysis)	√	√	X	X
mandibular teeth score (in 20 teeth analysis)	X	√	√	√
buccal surface score (in 20 teeth analysis)	X	X	√	X
lingual surface score (in 20 teeth analysis)	√	√	X	√

Table 4.33 illustrates the consistency of changes for gingival and bleeding indices over the time periods (weeks 0 - 3, 0 - 6 and 3 - 6), when only one data set was analysed. Only the TTO mouthwash 6 week data were used in this comparison. As there were only 2 preparations tested in the TTO mouthwash 6 week study, the relationship of the preparations to each other and the changes over time are used. A "+" indicates an increase in index scores; "+ +" indicates that preparation increased by a greater amount than the other preparation in that index score. A "-" indicates a decrease in index

scores and " - - " indicates that preparation decreased by a greater amount than the other preparation in that index score. A "0" indicates there was no detectable change.

Table 4.33 Pattern of changes in gingival and bleeding indices using different data sets.

	Gingival index			Bleeding index		
	wk 0-3	0-6	3-6	0-3	0-6	3-6
total mean score	- - -	- - -	- *0*	- +	- +	- +
mean score 28 teeth (average of 12 values)	- - -	- - -	+ + +	- +	- +	- - -
14 maxillary teeth	- -	- -	+ + +	- +	- +	+ + +
14 mandibular teeth	- - -	- - -	- 0	- +	- +	- - -
buccal surfaces (in 28 teeth analysis)	- - -	- - -	- - -	- +	- +	- - -
lingual surface score (in 28 teeth analysis)	- - -	- - -	+ + +	+ + +	+ + +	+ -
mean score 20 teeth	- - -	- - -	+ + +	- +	- +	- - -
10 maxillary teeth score	- - -	0 -	+ + +	- +	- +	+ + +
10 mandibular teeth score	- - -	- - -	- 0	- +	- - -	- - -
buccal surface score (in 20 teeth analysis)	- - -	- - -	- - -	- +	- +	- - -
lingual surface score (in 20 teeth analysis)	- - -	- - +	+ + +	+ + +	+ + +	+ -

In every cell in Table 4.33, the top symbols apply to the TTO mouthwash and the bottom symbol applies to the placebo. In this comparison, consistency in the pattern of changes in index scores was compared with the other data sets was sought.

CHAPTER 5

DISCUSSION

This study tested the efficacy of oral hygiene products that had been formulated by two separate companies. While the study did not develop new methods to evaluate the products, the analyses of the data have provided useful information regarding the design and analysis of similar studies in the future. In addition, the study has highlighted the potential problems that can occur when the contents of the products tested are controlled by industry. This will be discussed in further detail below.

Subject related issues

Subjects were recruited from the tertiary education campuses located in the city of Adelaide; they were recruited by leaflets, posters and advertisement in the University publications. The 4 day plaque growth studies were designed for 32 subjects in the chlorhexidine toothpaste (Part 1) and 30 in the TTO mouthwash (Part 2). A high dropout rate occurred in Part 2, (5 subjects failed to complete the study). The reasons for this ranged from forgetfulness to unforseen study commitments. Also, the review appointment was on Friday afternoons, and may have coincided with social events of the week. In Part 1 (chlorhexidine toothpaste study), the dropout rate was only 2. However, the difficulties encountered in the Part 1 study stemmed from the relatively higher number of dental studcnts participating. Unlike the more theoretically based courses, where there is usually an hour within an afternoon or morning session which is 'private study time'; the dental students were usually committed to clinics or laboratories for the entire session making it difficult to slot in review times.

The effects of the preparations were obviously different on teeth of high plaque formers (Figure 4.2). In contrast, an inspection of the colour slides of plaque-disclosed teeth in some subjects, the labial surfaces were found to be free of plaque for all preparations. These differed from the plaque distribution on teeth of low plaque formers, who exhibited some plaque formation with the placebo preparation (Figure 4.3). Some subjects had clearly not complied with the protocol of the study and had apparently brushed their teeth. There were 3 suspected non-compliant subjects in Part 1 and 6 in Part 2. The data analyses had not excluded these subjects.

Analysis of the data without the 'non-compliant' subjects was conducted and revealed no changes in ranking or statistical significance. Therefore, the details of the revised calculations were not included in this thesis.

In Part 3 (TTO mouthwash 6 week study), the sample size was smaller than the planned 30 subjects in the test and control groups (with only 24 and 25 subjects respectively) due to the low incidence of chronic gingivitis in the volunteers and high dropout rate during the study.

Study design related issues

Blind randomiscd controlled clinical trials are fundamental for scientific evaluation of products for the prevention of disease (Yates et al. 1998). Ideally, preparations should be in liquid form because a liquid can exert a more uniform plaque inhibitory action in different parts of the mouth, as there is no doubt about the distribution of the active agent throughout the solution. However, the model has been extensively used to test toothpastes slurries because it overcomes the tooth brushing variable (provided that

subjects refrained from brushing). The evaluation of the plaque inhibitory activity of a toothpaste should not only rely on the results of a 4 day plaque growth study but be followed up by a long term home use study when the product should be used as it was designed, ie. as a toothpaste on a toothbrush (Binney et al. 1992).

The power to detect differences in cross-over studies, where each subject is their own control, is considerably larger than in parallel studies (where there are different subjects testing different preparations).

Issues associated with Part 1 Chlorhexidine toothpaste study

The rationale for testing plaque inhibitory products on high plaque formers is that plaque inhibitory activity can be more clearly observed and scored (Gjermo et al. 1974). Double blindness could not be maintained for the chlorhexidine mouthwash in this study because it was the only mouthwash form while the other preparations tested were toothpastes. However, the subjects were unaware of the composition of any of the issued preparations. The three toothpastes were trialed in double-blind conditions.

Issues associated with Part 2 TTO mouthwash study

The use of the preparations were not evenly balanced between time because of the high subject dropout rate. Therefore, there were large differences in the number of subjects who used a particular preparation at each time. For example at time 4, more subjects used the 'positive control' than the other three preparations. If at time 4, some environmental factor caused the scores to be high, then the total score for the positive control would have been artificially elevated (relative to the other three preparations). This imbalance may result from interaction of preparation and time, which would not be

a true reflection of the activity of the preparations. There appears to be no conclusive evidence that some environmental factor may have skewed the results, however, the imbalance should be acknowledged.

The most significant and disturbing issue is that the supplier had added other plaque inhibitory agents to the test product without informing the trial conductor. The suppliers only informed the researcher that cetyl pyridinium chloride (CPC) and triclosan had been added to the TTO mouthwash. No information about the concentration of these agents were given. As a consequence, no controls for the other plaque inhibitory agents mixed with the TTO were incorporated into the study design, rendering the study incapable of establishing the plaque inhibitory effects of TTO alone. The Human Research Ethics Committee has since been alerted to this situation.

A positive control and a negative control should be included in a clinical trial to polarise the results so that the test product's effects falls somewhere between the two controls. Another serious complication of a chemical nature in this part of the study involved the supply of an inactive chlorhexidine mouthwash positive control. Prior to sending it to the researcher, the supplier had added a food colouring to the chlorhexidine mouthwash so that it matched the colour of the other preparations. The supplier had not conducted minimal inhibitory concentration tests (MIC) on the modified chlorhexidine mouthwash prior to sending it for trial. The anionic groups on the food colouring had effectively inactivated the chlorhexidine mouthwash, demonstrating how readily chlorhexidine can be inactivated. Therefore, this part of the study did not have a valid positive control. The TTO mixture could only be evaluated relative to the commercially available

Listerine® and the placebo (which we assume to contain no active plaque inhibitory agents).

Listerine® is a non-prescription, non-ionic broad spectrum antimicrobial mouthrinse, whose active ingredients are essential oils of thymol, methol, eucalyptol and methyl salicylate. It differs from chlorhexidine, where the mild staining associated with its long term use is easily removed with toothbrushing; and its taste is not as unpleasant as chlorhexidine, and it exhibits moderate plaque inhibition (Schaeken et al. 1994). This present study reported Listerine® as the most disliked preparations, in the absence of an active chlorhexidine mouthwash. This product is not as effective as chlorhexidine mouthwash.

5.1 PROPOSED PLAQUE INDEX

Index related issues

The plaque index used was not sensitive enough to accurately reflect the amount of plaque which usually covered less than a third of a tooth crown. The index used had only 3 scores to discriminate between the variations of plaque covering a third of the tooth crown. Neither the intensity nor the thickness of the plaque was accounted for by the Quigley and Hein plaque index which is not appropriate when small amounts of plaque are present. Even when plaque coverage exceeded a third of the crown of the tooth, the qualitative parameters of intensity or sparseness of plaque were not able to be scored.

A Tri-facet Plaque Index (TPII) is proposed. This index is a modification of several widely used indices. Three elements are proposed: each having its own value ie. a scale of 0-5 for coverage; and 1-5 for intensity and sparseness.

In terms of coverage, the Quigley and Hein (1962) index adequately addresses this issue. The quality of plaque accumulation on each third of a tooth crown is addressed by the other two elements of the proposed TPII.

Table 5.1 Coverage of plaque on tooth crown. (Quigley and Hein 1962)

0	no plaque
1	flecks of stain at gingival margin
2	definite line of plaque at gingival margin
3	gingival third of surfaces
4	two thirds of surface
5	> two thirds of surface

The intensity of the disclosed plaque can be scored using a modification of Sanz et al (1994) stain index which provided a range of intensity for stain / discolouration. In a similar way, the intensity of disclosed plaque can be scored.

Table 5.2 Intensity of disclosed plaque colour

1	very light pink (pellicle like appearance)
2	light pink
3	pink
4	dark pink
5	very dark pink

However, disclosed plaque after the use of chlorhexidine mouthwash usually appears to be dark pink; and may result in a higher TPII score. The intensity rating is mainly to distinguish between thick plaque (usually at least pink) and that of stained pellicle (which is usually light pink).

Sparseness can be scored taking into account the distribution and thickness of the plaque.

Table 5.3 Sparseness of plaque distribution

1	few flecks of plaque
2	equal amount of plaque and space within an area
3	a few spaces within the plaque mass
4	no spaces in the plaque mass
5	dense thick plaque with no spaces

These three elements can be analysed independently or combined. A combined score (of the three elements) would represent a better qualitative picture of the plaque accumulation in clinical and statistical terms. Admittedly, there is still an element of

subjectivity in this TPII but it provides more structure for plaque assessment than do the available indices.

This proposed TPII index can be illustrated by referring to Figure 4.4. Consider the plaque accumulation on the lower left central incisor (FDI notation 31).

Table 5.4 The plaque score for each preparation on the labial surface of tooth 31:

Preparations	coverage	intensity	sparseness	Total TPII
chlorhexidine toothpaste 1	4	2	4	10
placebo	4	3	4	11
Colgate Total®	4	4	4	16
0.12% chlorhexidine mouthwash	4	5	2	11

The column labelled 'coverage' reflects the plaque score using the Quigley and Hein (1962) index. This index does not distinguish between the different quality of plaque present with the four different preparations. With the TPII, the different quality of plaque is reflected in the total TPII score. This proposed TPII needs to be tested against established indices prior to its use in clinical studies.

A review of the literature revealed that the plaque index scores in this study were generally high compared to other studies using similar indices.

Table 5.5 Mean plaque scores of chlorhexidine mouthwash in other studies

Studies	Plaque Index	Mouthwash	Mean total plaque score
(Addy et al. 1989)	Greene & Vermillion	Chx 0.2% triclosan	0.1 0.2
(Binney et al. 1992)	Turesky	Chx 0.2%	1.6
(Binney et al. 1995)	Turesky	Chx 0.2%	1.64
(Binney et al. 1997)	Turesky	triclosan	2.14
(Jenkins et al. 1993)	Turesky	triclosan	1.2
(Jenkins et al. 1994a)	Turesky	Chx 0.05%	2.1
(Jenkins et al. 1994b)	Turesky	Chx 0.2	0.15
(Moran et al. 1992)	Quigley & Hein	Chx	2.9
(Moran et al. 1994)	Quigley & Hein	Chx	0.8
(Moran et al. 1995)	Turesky	Chx 0.2%	1.8
(Mendieta et al. 1994)	Quigley & Hein	Chx 0.12%	1.5
(Renton-Harper et al. 1996)	Turesky	Chx 0.12%	1.7
(Smith et al. 1995)	Turesky	Chx 0.12%	2.1
This study	Quigley & Hein	Chx 0.12%	2.5

Plaque scores may appear 'high' because of the nature of the index which scores small amounts of plaque relatively highly. In other words, the plaque scoring system can 'inflate' plaque scores where actual plaque amounts are quite low due to the distribution of the plaque because the index only addresses coverage of plaque. There is also an element of subjectivity when dealing with plaque amounts covering less than a third the tooth crown. Plaque scores may have been 'rounded' up, rather than down.

Preparation related issues

In the chlorhexidine toothpaste study, the process of converting lengths of toothpaste into a suspension or slurry is full of variation and difficulties. In the first instance, the subjects were instructed to add water to a pre-marked level on the bottles containing the lengths of toothpaste. The variation here is the amount of water added. When more water is added, the concentration of the preparation decreases, and the opposite when less water is added, but the final dose is the same. Water levels vary if the level is not

assessed at eye-level, or when the bottle is not placed on a flat surface, or when the pre-marked level is above the meniscus rather than below.

The second source of variation is the water temperature. The higher the temperature, the greater the rate of dissolution of a paste. A subject who used warmer water may incorporate more of the toothpaste into a slurry than a subject who used cold water. The third source of variation relates to the vigour with which the toothpaste and water was stirred, and shaken in the bottle. A subject who stirred and shook the mixture more vigorously may have incorporated more of the toothpaste into the slurry than a subject who was more gentle. Although the stirring rods were standardised and the subjects received the same instructions about the duration of stirring and shaking, there was no standard scale of vigour which could be standardised within and between subjects.

These sources of variation have not been discussed in the literature; they could be overcome by standardised ultrasonic homogenised pre-mixing prior to their issue. While pre-mixing effectively incorporates the toothpaste into a slurry, the dilution of the preservative is a health concern. Minimal inhibitory concentration tests carried out by Hamilton Laboratories have shown that bacterial growth could be significant in a pre-mixed solution. The amount of additional preservatives which would be required to stop bacterial growth in thc pre-mixed solution may interfere with any plaque inhibitory action of the product. Therefore, pre-mixed toothpastes were not used in this study.

Variations can also occur in the rinsing procedure, which was explained and demonstrated during the issue of the preparations. The subjects were instructed to rinse for 60 seconds and to move the solution around their mouth with their tongues. The

first source of variation between subjects is the duration of rinsing. The second source of error is in the technique of tongue movement to counteract the effect of gravity. Subjects who were less diligent in distributing an active agent to the buccal posterior regions could have had a higher plaque index score than those who were more diligent in tongue movements to the area and did not allow the pooling of preparations in the floor of the mouth. Variations in the rinsing process could be overcome by supervised rinsing which would require a greater commitment by the subjects (by attending 10 times as opposed to 2 for each preparation) and additional staffing to supervise the rinsing. The variations from the protocol could be logged in a diary, which would then make it possible to partially account for these variables, provided the subjects were diligent about keeping the log (Eaton et al. 1997).

Since rinsing was unsupervised, variations must have occurred between subjects and within subjects on different days. It is not possible to estimate or quantify this variable, which may also be common to other studies. However, since this was a cross-over study, each subject acted as their own control and it was assumed that each subject had a consistent rinsing behaviour.

5.2 COMPARISON BETWEEN DIFFERENT DATA TYPES

Collecting information about plaque levels, staining and gingival health is a time consuming process in large scale clinical trials. Reducing either the number of teeth or the tooth surfaces scored (or both) would make trials easier to carry out, provided the data type (teeth or surface) chosen for scoring gave data that are reflective of the total mean score (all teeth present). Therefore, it was decided to compare the analyses of the data sets for 28 and 20 teeth, lingual and buccal surface, and maxillary and mandibular teeth.

28 and 20 teeth analysis

Poor visual access by the researcher to molar teeth, especially the buccal surfaces of maxillary teeth, and the lack of access by the preparations to the same area also influenced the need for analysing different data sets. The uneven distribution of the plaque inhibitory agents may explain the variations in plaque inhibitory effects throughout the mouth, with certain sites receiving limited dose of the preparations (Addy and Hunter 1987).

Numerous studies of plaque accumulation have been limited to 20 non-molar teeth (De Paola et al. 1989; Overholser et al. 1990; Joyston-Bechal and Hernaman 1993; Lindhe et al. 1993; Kanchanakamol et al. 1995; Saxer et al. 1995; Triratana et al. 1995); and some have used fewer teeth (Grossman et al. 1989; Yates et al. 1993; Bollmer et al. 1995; Eaton et al. 1997). The advantages of scoring only 20 teeth (the non molar teeth) as opposed to 28 teeth include better visual access, and quicker scoring process. Subjects have an easier task of distributing the preparations to only as far distally as the premolar

teeth. There would be no need for them to consciously use their tongue to move the toothpaste slurries to the buccal molar surfaces to distribute preparations uniformly.

Tables 4.32 and 4.33 compare the ranking of preparations between mean scores for plaque, stain, gingival and bleeding indices for different data sets (ie. maxillary teeth only, or lingual surfaces only etc). When there was consistency and agreement of a data set with the overall mean scores, a 'tick (√)' has been shown. If the analyses showed a different ranking of preparations for that data set, then a 'cross (X)' appears. Only the chlorhexidine toothpaste and TTO mouthwash 4 day plaque growth data have been used in this comparison. For example, in the study involving chlorhexidine toothpaste, the analysis of the mean plaque score for all teeth / surfaces showed that the plaque index score (from the lowest to the highest) was:

1. chlorhexidine mouthwash,
2. chlorhexidine toothpaste,
3. Colgate® Total and
4. placebo.

When the plaque scores of only the 14 maxillary teeth (both buccal and lingual surfaces) were analysed, the same ranking was apparent.

Plaque index

The ranking of preparations in relation to the mean plaque score of buccal and lingual surfaces of 28 teeth for chlorhexidine toothpaste and TTO mouthwash was the same as the analysis of:

- 14 maxillary teeth of 28 teeth analysis, buccal and lingual surfaces,
- 14 mandibular teeth of 28 teeth analysis, buccal and lingual surfaces,

- lingual surfaces of 28 teeth analysis,
- mean plaque score of 20 teeth analysis,
- 10 maxillary teeth of 20 teeth analysis, buccal and lingual surfaces,
- lingual surfaces of 20 teeth analysis (ie. 10 maxillary and 10 mandibular teeth).

Stain index

The ranking of preparations in relation to the mean stain score for chlorhexidine toothpaste and TTO mouthwash was in agreement with analysis of:

- 14 mandibular teeth of 28 teeth analysis, buccal and lingual surfaces,
- mean stain score of 20 teeth analysis, buccal and lingual surfaces,
- 10 mandibular teeth of 20 teeth analysis, buccal and lingual surfaces,

Future studies may be able to use the comparison above, and score stain index using the different data sets to effectively obtain the results of scoring buccal and lingual surfaces of 28 teeth.

Future studies may be able to use the information above for plaque and stain scores to streamline data collection.

In the chlorhexidine toothpaste study, the plaque index scores for the maxillary molars (especially the buccal surfaces) were consistently high for all preparations, probably due to anatomical and physiological sheltering of the area from the preparations. By removing this group of consistently high scoring group of molar teeth (Addy and Hunter 1987), we can consider the changes of plaque accumulation on teeth which were exposed most consistently to the preparation, and more accurately assess the plaque

inhibitory activity of the preparations. This was observed in the analyses of 28 and 20 teeth in this study.

The strength of the effect (in terms of p values) of the preparations on plaque scores was similar for both analyses. The higher plaque scores of the molars effectively inflated the 'mean' score for the analyses of 28 teeth. On the other hand, the low stain index score of the molar teeth effectively dampened the effects of preparations; the stain scores were higher for the analyses of 20 teeth in comparison to the 28 teeth, because most staining occurred in the non molar teeth. Stain scores increased from the 28 teeth analysis to the 20 teeth analysis and may reflect the absence of the deflationary effects of the low scoring molar teeth on the score. The ranking order of the preparations remained the same.

Buccal and lingual surfaces

The lingual surfaces, especially the palatal surface of the maxillary teeth had lower plaque scores when compared to the buccal surfaces. This may be due to the natural cleaning by tongue and mastication (Addy and Hunter 1987). However, the buccal surfaces are more prone to unintentional abrasion of plaque (especially in the anterior region), and are more vulnerable during mastication and ingestion of acidic drinks. In terms of stain, higher stain scores were shown on the lingual surfaces of the mandibular teeth in the chlorhexidine toothpaste study and during week 3 to 6 period in TTO mouthwash 6 week study. This may be due to pooling of all preparations in the floor of the mouth.

In Part 3 TTO mouthwash 6 week study, during the period week 3 to 6, the TTO preparation appeared to result in a greater decrease in plaque index score on the buccal surfaces, than the placebo preparation, which caused an increase in the mean buccal plaque score and a small decrease in lingual plaque score. As subjects were requested to refrain from brushing 24 hours prior to their review appointment, non-compliance with this instruction could have resulted in decreased plaque levels. If this non-compliance was greater in the TTO group, it might explain our results. Otherwise, the decrease in plaque index score on the buccal surfaces were unexpected, considering the effects of mouthwashes would be expected to be more pronounced on the lingual surfaces due to 'pooling' in the floor of the mouth. This is illustrated in Table 5.1, where the scoring of 14 mandibular teeth resulted in the same ranking of preparations as the total mean score.

In Part 3 TTO mouthwash 6 week study, during the week 3 - 6 period, there was a significant difference between the stain score of the buccal and lingual surfaces of mandibular teeth. Stain score changes over this period were significant on the lingual surfaces between the TTO and placebo preparations, this may also explain the effects of gravity in terms of pooling of mouthwash in the floor of the mouth, prolonging the exposure of the lingual surfaces of the teeth to the active agents.

In Part 3 TTO mouthwash 6 week study, within the TTO preparation, there was a significant difference in plaque scores between the buccal and lingual surfaces during week 0-3. That is, the difference between the buccal and lingual scores were greater than twice the standard deviation of the difference. The bleeding index analysis between week 3 and 6, (in contrast to the analyses over the other time periods), the placebo preparation had a greater decrease in bleeding score compared with the TTO

preparation. The greater decrease here can in part be attributed to the change from very poor oral hygiene practices (pre-clinical trial) to tooth brushing and rinsing twice a day during the trial.

Gingival index

When the total mean gingival score changes was considered, TTO group decreased in all three time periods; and the placebo decreased in two time periods. This pattern of change is also evident in the data from 14 mandibular teeth. Therefore, these two sets of data (total mean gingival score and score from 14 mandibular teeth) are consistent with each other.

The only data set which showed agreement with the mean score data over the three time periods was 14 mandibular teeth data set. Future studies may therefore use the gingival scores of 14 mandibular teeth and be able to extrapolate the results to the total mean gingival scores.

Bleeding index

No data sets showed agreement with the mean bleeding index score over all three time periods. This means that a maximum of 28 teeth, both buccal and lingual surfaces need to be scored to best reflect the effects of preparations.

5.3 CHLORHEXIDINE 4 DAY PLAQUE GROWTH

Issues related to the chlorhexidine toothpaste study

Placebo toothpastes are difficult to formulate due to the fact that some toothpaste ingredients may have plaque inhibitory effects (Barkvoll et al. 1989; Marsh 1991). In this study, the placebo was the base of the chlorhexidine toothpaste which contained no active plaque inhibitory agent and performed accordingly. The plaque inhibitory effects of the liquid product (0.12% chlorhexidine mouthwash) was marked and was much more pronounced than the other three preparations. This result conforms with the well documented plaque inhibitory effect of chlorhexidine. However, the plaque index scores were not zero.

Colgate Total® represented the commercially available option and its plaque inhibitory agent was triclosan (2,4,4'trichlora-2'-hydroxydiphenyl ether) which is a non-ionic broad spectrum antimicrobial agent with activity against Gram positive and Gram negative bacteria (Walker et al. 1994). It has little substantivity in the oral cavity. Triclosan has been reported to have limited plaque inhibitory activity in aqueous solution (Jenkins et al. 1994b), and significant less plaque inhibitory activity than chlorhexidine mouthwash (Jenkins et al. 1994a). In a 4 day study, triclosan had an increased chemical plaque inhibition when compared to a placebo and fluoride toothpaste (Binney et al. 1997); and in a few long term home use studies (Lindhe et al. 1993; Palomo et al. 1994; Schaeken et al. 1994; Renvert and Birkhed 1995). The long term studies reported conflicting results in terms of anti-plaque activity (Saxton et al. 1993; Svatun et al. 1993; Palomo et al. 1994; Smith et al. 1994; Renvert and Birkhed 1995). Chlorhexidine

toothpaste has been shown to be more effective in plaque inhibitory activity than placebo preparations in other studies (Gjermo and Rölla 1971; Russell and Bay 1978).

The plaque inhibitory activity of the chlorhexidine mouthwash was far superior than various formulations of its toothpaste counterpart (Addy et al. 1989). The investigation into the plaque inhibitory effects of chlorhexidine containing toothpaste span the last two decades (Eriksen and Gjermo 1973; Johansen et al. 1975; Russell and Bay 1978; Dolles et al. 1979; Jenkins et al. 1990; Maynard et al. 1993; Yates et al. 1993; Sanz et al. 1994). In one study, there was no statistical difference between the chlorhexidine containing toothpaste and triclosan plaque inhibitory activity (Jenkins et al. 1990); and in another no difference in plaque inhibitory activity between chlorhexidine containing toothpaste and placebo (Johansen et al. 1975). In the few long term studies published, chlorhexidine containing toothpaste had greater plaque inhibitory effects and lower gingival score than a sodium monofluoro phosphate toothpaste (Sanz et al. 1994); lower plaque and gingivitis levels than placebo (Yates et al. 1993); and lower plaque and gingival scores than the placebo (Russell and Bay 1978). In this study, the plaque inhibitory effects of chlorhexidine toothpaste and triclosan were similar to that reported by Jenkins et al (1990). However, the comparison between all versions of chlorhexidine toothpaste and the placebo was statistically different, and was in contrast to the results of Johansen et al (1975). The chlorhexidine toothpastes tested in this study appeared to have a greater plaque inhibitory effect when compared to the other studies. However, there is little information on the formulations of the placebo preparations in studies and standardisation for comparison is difficult.

In this study, the stain score of the chlorhexidine toothpaste was not as high as the chlorhexidine mouthwash. As the stain index score was positively correlated to the use of chlorhexidine containing toothpaste (Eriksen and Gjermo 1973), the stain results in Part 1 may have given some information on the relatively low bioavailability of chlorhexidine in the toothpaste. Stain is an adverse side-effect of chlorhexidine mouthwash. One of the objectives of the new chlorhexidine toothpaste formulations was to decrease this side-effect. The amount of staining correlates with the substantivity of chlorhexidine and its plaque inhibitory activity. A reduced stain score was associated with a reduction in chlorhexidine bio-availability (Mendieta et al. 1994). Clinical studies of the influence of chlorhexidine concentration on staining are few and poorly controlled (Flötra et al. 1971; Cumming and Löe 1973; Lang et al. 1982). Staining was only obvious with the chlorhexidine mouthwash in this study. The subjects' diets were not standardised, and despite the subjects being their own control in a cross-over study, their diet may have varied eg. more coffee during examination periods as opposed to term time. The staining propensity of three other preparations may have had insufficient time to be apparent in 4 days. In concurrence with Sanz et al (1994), significantly less staining was found with chlorhexidine toothpaste compared with chlorhexidine mouthwash. The chlorhexidine toothpastes tested in this study may have been formulated to reduce staining at the expense of some loss of plaque inhibitory activity (Addy et al. 1991). The chlorhexidine toothpaste formulation aimed at lower staining propensity, may gain greater social acceptance. In this way, the commercial viability of this product increases regardless of the fact that its plaque inhibitory activity may not be similar to chlorhexidine mouthwash. The chlorhexidine toothpastes may provide clinical benefits in a long term study. A longer term home use study would more conclusively

determine and confirm the staining ability of the toothpaste preparations, and especially the chlorhexidine toothpaste.

Unpleasant taste is another distinct adverse side effect of chlorhexidine mouthwash. Therefore, in the incorporation of chlorhexidine into a toothpaste formula, taste needed to be assessed. The subjects did not report the 'unpleasant' taste of chlorhexidine in the chlorhexidine toothpaste. This observation may further indicate that the chlorhexidine in the toothpaste preparation was not bioavailable.

The chemistry of toothpastes are more complicated than mouthwashes. There are potential perils of extrapolating results from the use of active ingredients in simple mouthwash formulations to effects achievable with complex vehicles such as toothpastes, because many toothpaste ingredients also possess antimicrobial and plaque inhibitory properties (Addy et al. 1989).

5.4 TEA TREE OIL 4 DAY PLAQUE GROWTH

In this study, Listerine® was shown to significantly reduce plaque formation over 4 days when compared to the placebo. The plaque inhibitory activity of Listerine® has been extensively researched. In a 6 week and a 6 month trial, Listerine® was reported to inhibit plaque and gingivitis when compared to a hydroalcohol control and saline (De Paola et al. 1989; Ross et al. 1989) respectively. However, in a 6 month trial, Listerine® was less effective in inhibiting plaque when compared to Peridex® (0.12% chlorhexidine) (Overholser et al. 1990). Surprisingly, these products had similar effectiveness in inhibiting gingivitis in the same trial.

5.5 TEA TREE OIL 6 WEEK EFFECTS ON ORAL HEALTH

A 6 week clinical trial was selected to test the long term effects of TTO mouthwash for several reasons. Firstly, 6 weeks duration is long enough for the resolution or exacerbation of gingivitis to occur (Jenkins et al. 1993). Clinical trials of 4 weeks duration have also been used to determine the effectiveness of oral hygiene products (Baab and Johnson 1989; Schaeken et al. 1994; Hase et al. 1995). Secondly, two review appointments, one at week 3 and the other at week 6, provided two sets of data to compare with the baseline records. The mouthwash containing TTO reduced plaque formation but did not result in improved gingival health, probably because of the low baseline gingival and bleeding index scores. A shortcoming of this study was that no positive control in the form of a chlorhexidine mouthwash was used because the one industry supplied was inactive. In addition, there were no controls for the other active agents (ie. CPC and triclosan) that had been added to the TTO mouthwash by the supplier without informing the researcher.

In long term home use studies, variation in the time between the last toothbrushing and the plaque scoring appointment could influence the data (Renton-Harper et al. 1998). The timing of the last session of brushing has been standardised in some studies of oral hygiene products. Previous studies favoured brushing the morning prior to the day of the review appointment (Forgas-Brockmann et al. 1998; Renton-Harper et al. 1998; Van der Weijden et al. 1998). Subjects in this study were requested to perform their last brush/rinsing in the morning prior to the day of their review appointment. A confounding factor is toothbrushing technique. To avoid altering this dependent variable, many authors recommend that no toothbrushing instruction be given (Gjermo

and Rölla 1971; Grossman et al. 1989; Jenkins et al. 1993; Joyston-Bechal and Hernaman 1993; Yates et al. 1993; Palomo et al. 1994; Sanz et al. 1994; Bollmer et al. 1995; Saxer et al. 1995).

The decrease in plaque index scores observed in the test group could be attributed to either the Hawthorne effect (Binney et al. 1997), the therapeutic effects of the fluoride toothpaste issued to subjects (Yates et al. 1998), and/or the actual plaque inhibitory effects of the test agent. In the long term home use clinical trial, the imprecise variables such as the toothbrushing and Hawthorne effects, could have influenced the outcome. The Hawthorne effect occurs when subjects are conscious of their participation in an 'experiment' and may alter their behaviour (and in this case it is tooth-brushing behaviour / efficacy). This alteration of behaviour, be it for better or worse, effectively produces a change in the dependent variables and could jeopardise the validity of the study (Darby and Bowen 1980). The other component of the Hawthorne effect is the mentality of the volunteers who enrol in a clinical trial; they may be consciously intending to improve their oral hygiene status (Lindhe et al. 1993). In this study, the gingival score decreased for both the TTO and placebo preparations perhaps as a result of the Hawthorn effect, brushing teeth twice a day (as opposed to their usual oral hygiene practices prior to participation in this study), the fluoride toothpaste or other plaque inhibitory agents in the mouthwash 'placebo'. It is not possible to quantify the effects of any of the elements on the parameters measured.

There were no significant changes in gingival health even though plaque scores decreased, perhaps because of the generally low levels of chronic gingivitis at baseline. The oral health of the majority of the subjects was generally good, with mild gingivitis

limited to only a few sites. The selection criteria for this study was a minimum of 6 bleeding sites. A statistically significant result may have been obtained if the subjects had a greater amount of chronic gingivitis at baseline.

Similar long term studies in the literature have had a Gingival Index score of 0.5 (Jenkins et al. 1993) to 1.95 (Overholser et al. 1990) as the selection criteria. The most common minimum gingival index score was 1.0 (Kanchanakamol et al. 1995; Triratana et al. 1995; Binney et al. 1996). Some studies overcame the need for a minimum gingival index score by stratifying their baseline subjects for each preparation tested (Grossman et al. 1989; Palomo et al. 1994). Other studies were non-specific in their gingival health criteria by selecting subjects who 'showed signs of gingivitis' (Joyston-Bechal and Hernaman 1993; Lindhe et al. 1993; Renvert and Birkhed 1995). In contrast to the findings of this study, Sanz et al. (1994) reported that subjects with lower baseline gingivitis showed a greater response to the beneficial effects of a tested product over 6 months in reduction in bleeding sites than subjects with higher baseline gingivitis scores.

In the placebo group at weeks 3 and 6, it is interesting to note that despite an increase in plaque score (of 9% and 8% respectively), there were decreases in gingival score at the corresponding times (of 10%). This finding may be a result of effective removal of plaque at the gingival margin during the course of the study. The plaque accumulation which was seen in the plaque score, may only be a reflection of the amount of plaque formation during the 24 hours prior to the review appointment. In this case, the plaque score does not correlate well with the gingival score.

The 6 week trial was designed to measure the effects of the plaque inhibitory agents on plaque accumulation and gingival health; these effects can be measured on plaque and on gingivitis. This arrangement resembles the situation in real life, where the majority of the population experience some degree of plaque and gingivitis (Baelum et al. 1996). A product which decreases plaque accumulation and resolves previously established gingivitis is of more potential use than one which is only shown to reduce plaque levels.

This 6 week clinical trial was designed so that no prophylaxis was given after the baseline records had been taken (Baab and Johnson 1989; Ross et al. 1989; Jenkins et al. 1993; Lindhe et al. 1993; Saxer et al. 1995; Triratana et al. 1995; Yates et al. 1998). This protocol is in contrast to the following studies which had given subjects a prophylaxis at baseline (Lang et al. 1982; Addy and Hunter 1987; De Paola et al. 1989; Grossman et al. 1989; Overholser et al. 1990; Joyston-Bechal and Hernaman 1993; Yates et al. 1993; Kozlovsky et al. 1994; Palomo et al. 1994; Quirynen et al. 1994; Sanz et al. 1994; Bollmer et al. 1995; Hase et al. 1995; Kanchanakamol et al. 1995; Renvert and Birkhed 1995; Binney et al. 1996; Eaton et al. 1997). Further research into the effects of this prophylaxis on the subsequent scores would be indicated. A split mouth prophylaxis design would best demonstrate the effects of prophylaxis after baseline records had been taken.

Studies which used a scale and clean after baseline records were taken may show a positive effect on gingival tissues independent of the effects of active agents in mouthwashes. Where the trial designs includes a scale and clean and prophylaxis to be performed on the subjects following the recording of baseline measurements, then the results can artificially result in a 'more effective' active agent, as the plaque and calculus

present at baseline is removed. Gingival health is known to improve following a dental prophylaxis particularly if it incorporates supragingival and subgingival scaling; this is the basis of periodontal therapy (Lövdal et al. 1961). However, the opposing argument is that the long term effects of a dental prophylaxis may be negligible because following subgingival scaling, the microbiota re-establishes after a couple of months (Magnusson et al. 1984). Complete removal of subgingival calculus would effect the gingival health far more than supragingival calculus and plaque removal. The nature of the 'prophylaxis' in previous studies is generally not detailed.

Photographic slides of labial surfaces of teeth revealed that an unusual yellow film had developed on some subjects' teeth (Figure 4.9). In the TTO group, the yellow film occurred in approximately 50% of the subjects. In the placebo group, the yellow film was seen in about 35% of the subjects. It is interesting that this discolouration had not been detected during clinical examination and stain scoring. The mouthwash base may have contained a compound which was responsible for the formation of a yellow film in some subjects. The results also showed that long term use of TTO mouthwash was associated with increased staining. As other agents had been added to the TTO, it is not possible to say which components of the mouthwash contributed to the staining.

CHAPTER 6

CONCLUSION

Based on the findings of this study, the ranking of mean scores (a maximum of 56 scores) within plaque, stain and gingival indices can be obtained by analysing smaller data sets. However, the results of the mean bleeding index score was not reflected in any other smaller data sets.

A proposed plaque index (tri-facet Plaque Index) aims to better quantify the different qualities of accumulated plaque by including a rating for colour intensity and distribution sparseness in addition to area of crown coverage.

Part 1: Chlorhexidine 4 day plaque growth

Various formulations of chlorhexidine toothpaste can reduce plaque formation relative to a placebo, although they were significantly less effective than chlorhexidine mouthwash.

Part 2: TTO 4 day plaque growth

The TTO mouthwash mixture was as effective as Listerine in its plaque inhibitory activity; and both preparations were significantly more effective than the placebo. Unfortunately, because of the additional plaque inhibitory agents added to TTO the mouthwash and the inactivation of the positive control chlorhexidine mouthwash, this study did not provide scientifically valid information regarding the plaque inhibitory effects of TTO.

Part 3: TTO 6 week effects on oral health

The TTO mouthwash showed a decrease in plaque score, and a significant increase in stain score when compared to the placebo. Neither preparations showed significant differences with regard to gingival and bleeding index scores. As with Part 2, the addition of other plaque inhibitory agents to the TTO mouthwash rendered this study invalid.

CHAPTER 7

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Appendix I. Information sheet for Chlorhexidine 4 day plaque growth clinical trial

The University of Adelaide
Department in Dentistry

Information sheet for participants in the research project

"Effects of a chlorhexidine-containing toothpaste on dental plaque formation."

Purpose of this study

Chlorhexidine-containing mouthwashes are currently available in pharmacies, and are known to be effective in reducing dental plaque formation and preventing the development of gum inflammation (gingivitis). This study has been designed to test whether a newly formulated **toothpaste** containing the anti-bacterial agent chlorhexidine has beneficial effects on oral health.

In order to find this out, we need to measure the plaque build upwhen you rinse with a slurry of one of the following formulations: chlorhexidine toothpaste, non-chlorhexidine toothpaste, Colgate Total toothpaste or 0.12% chlorhexidine mouthwash.

What is involved?

At the first visit, you will have your teeth scaled and polished to remove plaque. You will be issued with one of the preparations listed above, together with written instructions.

The study will be conducted over a 4 day period. You will be asked to rinse twice a day with a preparation for 4 days. During this time, you will also be asked ***not*** to brush your teeth or to perform any other oral hygiene procedures, other than rinsing twice a day with the preparation issued.

At the next visit, your teeth and gums will be examined and photographed. A disclosing solution will be applied to the teeth to show where any plaque has formed. Your teeth will then be cleaned and polished. This appointment will take about 30 minutes.

What are the benefits to me?

Information from this study will be helpful in developing a new oral health care products which could have significant beneficial effects in keeping teeth and gums healthy. You will also be financially compensated to acknowledge your participation, and receive a free oral health assessment and scale and clean of your teeth.

Are there any risks?

The risks of being part of this study are considered to be very low. It is not anticipated that there will be adverse effects to the health of your gums and teeth. Your gums will become healthy again with the commencement of brushing following a professional cleaning. You may withdraw from this study at any time.

Any information you give us will be treated confidentially.

Please contact the following people if you have any questions:

Dr Adeline Chong
Mon-Fri 9-5
After hours

Dr Robert Hirsch
Mon-Fri 9-5
After hours

Appendix II. Consent form for Chlorhexidine 4 day plaque growth clinical trial

THE UNIVERSITY OF ADELAIDE

CONSENT FORM

See also Information Sheet attached.

1. I _____ (please print) hereby consent to take part in the research project entitled:

   THE EFFECTS OF CHLORHEXIDINE CONTAINING TOOTHPASTE ON DENTAL PLAQUE FORMATION, DEVELOPMENT OF GINGIVITIS AND ON CHRONIC GINGIVITIS

2. I acknowledge that I have read the Information Sheet entitled:

   EFFECTS OF A CHLORHEXIDINE-CONTAINING TOOTHPASTE ON DENTAL PLAQUE FORMATION

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED ..... **DATE**.....

NAME OF WITNESS..... **SIGNED**.....
(Please print)

DATE.....

I,.....have described to .....
(Please print)

the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

SIGNED ..... **DATE**.....

STATUS IN PROJECT .....

Appendix III. Schedule for Chlorhexidine 4 day plaque growth clinical trial

Chlorhexidine 4 Day plaque growth clinical trial schedule - randomised

Week beginning	Monday PM	Thursday PM
27/7/98		**mw A**
3/8/98	mw A	**mw B**
10/8/98	mw B	**mw C**
17/8/98	mw C	mw A
24/8/98	mw A	mw B
31/8/98	mw B	mw C
7/9/98	mw C	
14/9/98		
21/9/98		
28/9/98		
5/10/98		mw A
12/10/98	mw A	mw B
19/10/98	mw B	mw C
26/10/98	mw C	mw A
2/11/98	mw A	mw B
9/11/98	mw B	mw C
16/11/98	mw C	

Chlorhexidine 4 Day plaque growth clinical trial schedule - non-randomised

Week beginning	Monday PM	Friday PM
25/1/99	**mw D**	mw D
15/2/99	**mw E**	mw E
8/3/99	**mw F**	mw F

Appendix IV. Information sheet for TTO 4 day plaque growth clinical trial

The University of Adelaide
Department in Dentistry

Information sheet for participants in the research project

"Effects of a tea tree oil-containing mouthwash on dental plaque formation."

Purpose of this study

This study has been designed to test whether a newly formulated toothpaste containing the anti-bacterial agent tea tree oil has beneficial effects on oral health. Tea tree oil-containing mouthwashes are currently new in the market and we want to are to find out if they are effective in reducing dental plaque formation.

In order to find this out, we need to measure the plaque build up in people rinsing with one of the following formulations: 2% tea tree oil mouthwash, base mouthwash, 0.12% chlorhexidine mouthwash (an antiseptic agent), or Listerine mouthwash.

What is involved?

At the first visit, you will have your teeth scaled and polished to remove plaque. You will be issued with one of the preparations listed above, together with written instructions.

The study will be conducted over a 4 day period. You will be asked to rinse twice a day with one of the preparations for 4 days. During this time, you will be asked ***not*** to brush your teeth or to perform any other oral hygiene procedures, other than rinsing twice a day with the preparation issued.

At the next visit, your teeth will be examined and photographed. A disclosing solution will be applied to the teeth to show where any plaque has formed. Your teeth will then be cleaned and polished. This appointment will take about 30 minutes.
This procedure will be repeated 4 times, so that you will use all the different preparations.

What are the benefits to me?

Information from this study will be helpful in developing a new oral health care products which could have significant beneficial effects in keeping teeth and gums healthy. You will also be financially compensated to acknowledge your participation, and receive a free oral health assessment and scale and clean of your teeth.

Are there any risks?

The risks of being part of this study are considered to be very low. It is not anticipated that there will be adverse effects to the health of your gums and teeth. Your gums will become healthy again with the commencement of brushing following a professional cleaning. You may withdraw from this study at any time.

All the information you give us will be treated confidentially.

Please contact the following people if you have any questions:

Dr Adeline Chong
Mon-Fri 9-5
After hours

Dr Robert Hirsch
Mon-Fri 9-5
After hours

Appendix V. Consent form for TTO 4 day plaque growth clinical trial

THE UNIVERSITY OF ADELAIDE

CONSENT FORM

See also Information Sheet attached.

1. I _____ (please print) hereby consent to take part in the research project entitled:

   THE EFFECTS OF TEA TREE OIL-CONTAINING MOUTHWASHES AND TOOTHPASTES ON DENTAL PLAQUE FORMATION AND ON CHRONIC GINGIVITIS

2. I acknowledge that I have read the Information Sheet entitled:

   EFFECTS OF A TEA TREE OIL-CONTAINING MOUTHWASH ON DENTAL PLAQUE FORMATION

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED ..... **DATE**.....

NAME OF WITNESS..... **SIGNED**.....
(Please print)

DATE.....

I,..... have described to .....
(Please print)

the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

SIGNED ..... **DATE**.....

STATUS IN PROJECT .....

Appendix VI. Schedule for TTO 4 day plaque growth and 6week oral health clinical trials

Tea Tree Oil clinical trial schedule

Week beginning	Monday AM	Tuesday PM	Wednesday AM	Wednesday PM	Thursday PM	Friday PM
3/8/98	**mw A**					mw A
10/8/98	**mw B**					mw B
17/8/98	**mw C**	**tp A**	**tp B**	tp screen		mw C
24/8/98	mw A					mw A
31/8/98	mw B					mw B
7/9/98		tp A	tp B	tp screen		
14/9/98						
21/9/98						
28/9/98	mw C	tp A	tp B	**tp G**	tp screen	mw C
5/10/98		**tp D**	**tp E**	**tp F**		
12/10/98	mw A					mw A
19/10/98	mw B			tp G		mw B
26/10/98	mw C	tp D	tp E	tp F		mw C
2/11/98	mw A					mw A
9/11/98	mw B			tp G		mw B
16/9/98	mw C	tp D	tp E	tp F		mw C

Tea Tree Oil - 4 Day

The table above summarises the schedule of the visits of each group of subjects for the essential oil mouthwash. The mouthwash groups (mw) began their trial on 3/8/98, 10/8/98 and 17/8/98. In order to measure the plaque growth over 4 days, the subjects attended on the Monday (day 0) and were reviewed on the Friday (day 4) of the same week.

Tea Tree Oil - 6 Week

The subjects involved in the long term effects of the essential oil mouthwash over 6 weeks was represented by "tp". These subjects began their participation 18/8/98, 19/8/98, 30/9/98, 6/9/98, and two groups on 7/9/98 (which represented week 0). Then they were reviewed on week 3 and week 6. The 'tp screen' sessions were sessions allocated to select subjects with chronic gingivitis. Chronic gingivitis was assessed as the presence of colour change and bleeding on probing (GI 2 according to Loe 1967 gingival index).

Appendix VII. Information sheet for 6 week oral health clinical trials

The University of Adelaide
Department in Dentistry

Information sheet for participants in the research project

"Effects of a tea tree oil-containing mouthwash and toothpaste on oral health."

Purpose of this study

This study has been designed to test the long term effects of a newly formulated toothpaste containing the anti-bacterial agent **tea tree oil**. We want to find out if tea tree oil-containing toothpastes are effective in reducing dental plaque formation and preventing the development of gum inflammation (gingivitis).

In order to find this out, we need to measure whether tea tree oil has an effect in reducing the amount of gingivitis. We will examine the health of your gums when you brush and rinse with one of the following formulations: tea tree oil mouthwash, base mouthwash, tea tree oil toothpaste, base toothpaste, and Colgate Total toothpaste.

What is involved?

At the first visit, the level of plaque, gum inflammation and staining will be recorded. You will be issued with one of the preparations listed above, together with written instructions.

The study will be conducted over a 6 week period involving 2 more visits. You will be given a new toothbrush at the start of the study, and at Week 3. You will be asked to brush as you would normally for 6 weeks. You may also be given a mouthwash to rinse with, after brushing. At each of next 2 visits (Weeks 3 and 6), your teeth and gums will be examined and photographed. A disclosing solution will be applied to the teeth to show where plaque has formed.

On the last visit, your teeth will be cleaned and polished. The appointment at Week 3 will take about 15 minutes, and the final appointment will take about 45 minutes.

What are the benefits to me?

Information from this study will be helpful in developing a new oral health care products which could have significant beneficial effects in keeping teeth and gums healthy. You will also be financially compensated to acknowledge your participation, and receive two new toothbrushes, a free oral health assessment and scale and clean of your teeth.

Are there any risks?

The risks of being part of this study are considered to be very low. Your gingival health can only improve with the use of the anti-plaque agents in the formulations being tested here. Your gums will have a better chance of becoming healthy again at the end of the study following a professional cleaning. You may withdraw from this study at any time. All the information you give us will be treated confidentially.

Please contact the following people if you have any questions:

Dr Adeline Chong
Mon-Fri 9-5
Dr Robert Hirsch
Mon-Fri 9-5

Appendix VIII. Consent form for TTO 6 week oral health clinical trial

THE UNIVERSITY OF ADELAIDE

CONSENT FORM

See also Information Sheet attached.

1. I _____ (please print) hereby consent to take part in the research project entitled:

   THE EFFECTS OF TEA TREE OIL-CONTAINING MOUTHWASHES AND TOOTHPASTES ON DENTAL PLAQUE FORMATION AND ON CHRONIC GINGIVITIS

2. I acknowledge that I have read the Information Sheet entitled:

   EFFECTS OF A TEA TREE OIL-CONTAINING MOUTHWASH AND TOOTHPASTE ON ORAL HEALTH

3. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

8. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED ..... **DATE**.....

NAME OF WITNESS..... **SIGNED**.....
(Please print)

DATE.....

I,.....have described to .....
(Please print)

the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

SIGNED ..... **DATE**.....

STATUS IN PROJECT .....

Appendix IX. Analysis of variance (ANOVA) tables for Chlorhexidine 4 day plaque growth (Part 1)

MEAN PLAQUE INDEX

```
Error: pers
          Df Sum of Sq   Mean Sq F Value     Pr(F)
form:time  9  3.209505 0.3566117 1.67809 0.1603261
Residuals 20  4.250210 0.2125105
Bet subj  29  7.459715 0.2572316 4.30189 0.0000000

Error: time %in% pers
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       3  14.30025 4.766752 79.71834 0.0000000
time       3   0.15749 0.052497  0.87795 0.4564785
form:time  9   0.33241 0.036934  0.61768 0.7783627
Residuals 75   4.48462 0.059795
```

Analysis of 28 teeth

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
time:form  9  34.47958 3.831064 1.774224 0.1368335
Residuals 20  43.18581 2.159291

Error: time
     Df Sum of Sq   Mean Sq
time  3  2.588773 0.8629244

Error: pers:time
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       3  159.9649 53.32162 77.98288 0.0000000
time:form  9    3.4496  0.38329  0.56056 0.8249188
Residuals 75   51.2820  0.68376

Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1  201.1273 201.1273 826.6026 0.0000000
posn                2    0.1421   0.0711   0.2921 0.7467718
jaw                 1    0.6964   0.6964   2.8620 0.0909509
time:surf           3    5.1650   1.7217   7.0758 0.0001026
time:posn           6    1.7006   0.2834   1.1649 0.3226468
time:jaw            3    1.1319   0.3773   1.5507 0.1997148
form:surf           3    7.6715   2.5572  10.5096 0.0000008
form:posn           6    4.3159   0.7193   2.9563 0.0072010
form:jaw            3    2.1172   0.7057   2.9004 0.0339547
surf:posn           2   13.9121   6.9560  28.5883 0.0000000
posn:jaw            2   48.8955  24.4478 100.4766 0.0000000
surf:jaw            1  161.4477 161.4477 663.5254 0.0000000
time:form:surf      9    2.8739   0.3193   1.3124 0.2255190
time:form:posn     18    7.1585   0.3977   1.6345 0.0452800
time:form:jaw       9    3.3415   0.3713   1.5259 0.1335727
time:(surf:posn)    6    0.8141   0.1357   0.5577 0.7642274
time:(posn:jaw)     6    1.1285   0.1881   0.7730 0.5911661
time:(surf:jaw)     3    1.6185   0.5395   2.2173 0.0844291
form:(surf:posn)    6    0.8300   0.1383   0.5686 0.7556153
form:(posn:jaw)     6    1.4190   0.2365   0.9720 0.4427942
form:(surf:jaw)     3    1.2617   0.4206   1.7284 0.1593255
surf:posn:jaw       2   19.4977   9.7489  40.0663 0.0000000
Residuals        1219  296.6047   0.2433
```

Analysis of 20 teeth

```
Error: pers
          Df Sum of Sq  Mean Sq F Value     Pr(F)
time:form  9  52.10712 5.789680 1.77621 0.1363862
Residuals 20  65.19138 3.259569

Error: time
     Df Sum of Sq   Mean Sq
time  3  2.259896 0.7532986

Error: pers:time
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       3  195.7301 65.24335 70.83702 0.0000000
time:form  9    5.8106  0.64563  0.70098 0.7059842
Residuals 75   69.0776  0.92103

Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1  121.5297 121.5297 422.3544 0.0000000
posn                2    3.1571   1.5785   5.4859 0.0042478
jaw                 1   36.1000  36.1000 125.4590 0.0000000
time:surf           3    5.9234   1.9745   6.8619 0.0001388
time:posn           6    1.6485   0.2747   0.9548 0.4546906
time:jaw            3    1.5136   0.5045   1.7534 0.1543044
form:surf           3   13.4826   4.4942  15.6188 0.0000000
form:posn           6    1.4456   0.2409   0.8373 0.5410129
form:jaw            3    1.1765   0.3922   1.3628 0.2526007
surf:posn           2    4.0520   2.0260   7.0410 0.0009113
posn:jaw            2    9.2667   4.6333  16.1023 0.0000001
surf:jaw            1  106.8019 106.8019 371.1705 0.0000000
time:form:surf      9    6.0012   0.6668   2.3174 0.0138607
time:form:posn     18    5.1988   0.2888   1.0038 0.4522074
time:form:jaw       9    7.7453   0.8606   2.9908 0.0015674
time:(surf:posn)    6    0.4073   0.0679   0.2359 0.9648265
time:(posn:jaw)     6    1.9886   0.3314   1.1518 0.3299367
time:(surf:jaw)     3    0.8863   0.2954   1.0267 0.3798302
form:(surf:posn)    6    0.8972   0.1495   0.5197 0.7937386
form:(posn:jaw)     6    0.7954   0.1326   0.4607 0.8376486
form:(surf:jaw)     3    0.5305   0.1768   0.6146 0.6055966
surf:posn:jaw       2   18.6394   9.3197  32.3889 0.0000000
Residuals        1219  350.7593   0.2877
```

MEAN STAIN INDEX

```
Error: pers
          Df Sum of Sq   Mean Sq   F Value     Pr(F)
form:time  9  0.639459 0.0710511 0.2801708 0.9727218
Residuals 20  5.071981 0.2535991
Bet subj  29  5.711440 0.1969462 3.797168  0.0000018

Error: time %in% pers
          Df Sum of Sq   Mean Sq  F Value     Pr(F)
form       3  1.086463 0.3621545 6.982427 0.0003318
time       3  0.075811 0.0252702 0.487216 0.6921904
form:time  9  0.442208 0.0491342 0.947319 0.4900759
Residuals 75  3.889992 0.0518666
```

Analysis of 28 teeth

```
Error: pers
          Df Sum of Sq  Mean Sq   F Value     Pr(F)
time:form  9   6.68356 0.742618 0.2849033 0.9712163
Residuals 20  52.13123 2.606562

Error: time
     Df Sum of Sq   Mean Sq
time  3 0.7346644 0.2448881

Error: pers:time
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       3  10.53623 3.512075 6.868894 0.0003769
time:form  9   3.85604 0.428449 0.837958 0.5836767
Residuals 75  38.34761 0.511301

Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1    4.3707  4.37068  32.2816 0.0000000
posn                2   21.6743 10.83716  80.0427 0.0000000
jaw                 1   22.4584 22.45835 165.8763 0.0000000
time:surf           3    2.2466  0.74886   5.5310 0.0009005
time:posn           6    0.1933  0.03222   0.2380 0.9640502
time:jaw            3    0.3627  0.12089   0.8929 0.4441761
form:surf           3    0.2354  0.07845   0.5794 0.6285716
form:posn           6    2.0883  0.34804   2.5706 0.0176878
form:jaw            3    2.3352  0.77841   5.7493 0.0006633
surf:posn           2    3.9555  1.97775  14.6076 0.0000005
posn:jaw            2    9.3597  4.67987  34.5653 0.0000000
surf:jaw            1    5.0964  5.09637  37.6416 0.0000000
time:form:surf      9    0.6056  0.06729   0.4970 0.8772441
time:form:posn     18    2.2936  0.12742   0.9411 0.5276563
time:form:jaw       9    2.3506  0.26118   1.9290 0.0443884
time:(surf:posn)    6    1.4050  0.23417   1.7296 0.1106483
time:(posn:jaw)     6    1.0361  0.17269   1.2755 0.2655647
time:(surf:jaw)     3    0.8111  0.27036   1.9968 0.1126698
form:(surf:posn)    6    0.7279  0.12132   0.8961 0.4968886
form:(posn:jaw)     6    0.1399  0.02332   0.1722 0.9842713
form:(surf:jaw)     3    0.7294  0.24313   1.7958 0.1461414
surf:posn:jaw       2    2.9651  1.48256  10.9501 0.0000193
Residuals        1219  165.0430  0.13539
```

Analysis of 20 teeth

```
Error: pers
          Df Sum of Sq  Mean Sq   F Value     Pr(F)
time:form  9   9.98464 1.109404 0.2436228 0.9827783
Residuals 20  91.07559 4.553779

Error: time
     Df Sum of Sq   Mean Sq
time  3  1.017646 0.3392152

Error: pers:time
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       3  17.92232 5.974108 6.829046 0.0003942
time:form  9   9.09769 1.010854 1.155515 0.3358519
Residuals 75  65.61064 0.874808

Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1   13.2090 13.20903  63.7922 0.0000000
posn                2    9.3600  4.67999  22.6017 0.0000000
jaw                 1   49.1053 49.10533 237.1513 0.0000000
time:surf           3    4.3963  1.46545   7.0773 0.0001024
time:posn           6    0.2546  0.04243   0.2049 0.9753367
time:jaw            3    1.2831  0.42769   2.0655 0.1030211
form:surf           3    0.9816  0.32722   1.5803 0.1923796
form:posn           6    1.5129  0.25215   1.2177 0.2942875
form:jaw            3    3.1472  1.04908   5.0665 0.0017237
surf:posn           2    1.4974  0.74870   3.6158 0.0271838
posn:jaw            2    3.0312  1.51560   7.3195 0.0006920
surf:jaw            1   14.5839 14.58392  70.4322 0.0000000
time:form:surf      9    1.4861  0.16512   0.7974 0.6187503
time:form:posn     18    1.0053  0.05585   0.2697 0.9990379
time:form:jaw       9    4.5012  0.50013   2.4154 0.0102064
time:(surf:posn)    6    1.0400  0.17333   0.8371 0.5411976
time:(posn:jaw)     6    0.8380  0.13966   0.6745 0.6703350
time:(surf:jaw)     3    1.5955  0.53182   2.5684 0.0530124
form:(surf:posn)    6    0.5887  0.09811   0.4738 0.8281097
form:(posn:jaw)     6    0.1431  0.02384   0.1151 0.9946662
form:(surf:jaw)     3    1.9476  0.64921   3.1353 0.0247140
surf:posn:jaw       2    0.3763  0.18817   0.9088 0.4032975
Residuals        1219  252.4102  0.20706
```

Taste rating

```
Error: pers
          Df Sum of Sq  Mean Sq   F Value     Pr(F)
form:time  9   7.04762 0.783069 0.5587886 0.8142056
Residuals 20  28.02738 1.401369
Bet subj  29  35.07500 1.209483 1.737224  0.0296096

Error: Within
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       3  13.02500 4.341667 5.877122 0.0011673
time       3   4.54533 1.515109 2.050936 0.1139502
form:time  9  13.27415 1.474906 1.996515 0.0513218
Residuals 75  55.40552 0.738740
```

Appendix X. Analysis of variance (ANOVA) table for TTO 4 day plaque growth (Part 2)

MEAN PLAQUE INDEX

```
Error: pers
          Df Sum of Sq   Mean Sq  F Value      Pr(F)
form:tim   9  8.248264 0.9164737 2.123457 0.09485357
Residuals 15  6.473926 0.4315951
Total     24 14.722190 0.613425  7.440229 0.0000000
Error: time %in% pers
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       3  4.392021 1.464007 17.75705 0.0000000
tim        3  0.465953 0.155318  1.88386 0.1419420
form:tim   9  0.551152 0.061239  0.74277 0.6684019
Residuals 60  4.946792 0.082447
```

Analysis of 28 teeth

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value      Pr(F)
time:form  9  89.13878 9.904308 2.251634 0.07920845
Residuals 15  65.98081 4.398720

Error: time
     Df Sum of Sq  Mean Sq
time  3  7.539693 2.513231

Error: pers:time
          Df Sum of Sq  Mean Sq  F Value    Pr(F)
form       3  43.96991 14.65664 17.06801 0.000000
time:form  9   7.16905  0.79656  0.92761 0.508077
Residuals 60  51.52317  0.85872

Error: Within
                  Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf               1  142.1982 142.1982 466.1717 0.0000000
posn               2    3.5309   1.7655   5.7877 0.0031686
jaw                1    3.4045   3.4045  11.1609 0.0008663
time:surf          3    0.6022   0.2007   0.6581 0.5779830
time:posn          6    1.0167   0.1694   0.5555 0.7658940
time:jaw           3    0.5084   0.1695   0.5556 0.6444551
form:surf          3    3.8505   1.2835   4.2077 0.0057169
form:posn          6    3.9682   0.6614   2.1681 0.0438249
form:jaw           3    1.0200   0.3400   1.1147 0.3421024
surf:posn          2   16.8550   8.4275  27.6281 0.0000000
posn:jaw           2   22.7252  11.3626  37.2502 0.0000000
surf:jaw           1  144.2711 144.2711 472.9673 0.0000000
time:form:surf     9    5.3519   0.5947   1.9495 0.0420647
time:form:posn    18    7.8437   0.4358   1.4286 0.1094753
time:form:jaw      9    2.6379   0.2931   0.9609 0.4710629
time:(surf:posn)   6    0.5567   0.0928   0.3042 0.9348867
time:(posn:jaw)    6    1.1472   0.1912   0.6268 0.7089520
time:(surf:jaw)    3    2.6803   0.8934   2.9290 0.0327642
form:(surf:posn)   6    0.2488   0.0415   0.1360 0.9916072
form:(posn:jaw)    6    1.2537   0.2090   0.6850 0.6618039
form:(surf:jaw)    3    0.2503   0.0834   0.2735 0.8445406
surf:posn:jaw      2   13.6511   6.8255  22.3763 0.0000000
Residuals        999  304.7290   0.3050
```

Analysis of 20 teeth

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value    Pr(F)
time:form  9  123.2356 13.69284 2.035841 0.107439
Residuals 15  100.8883  6.72589

Error: time
     Df Sum of Sq  Mean Sq
time  3   10.7321 3.577367

Error: pers:time
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       3  66.30684 22.10228 16.79143 0.0000000
time:form  9   6.89364  0.76596  0.58191 0.8066992
Residuals 60  78.97700  1.31628

Error: Within
                  Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf               1   83.8730  83.8730 241.5204 0.0000000
posn               2   13.8440   6.9220  19.9326 0.0000000
jaw                1   30.6401  30.6401  88.2310 0.0000000
time:surf          3    0.7781   0.2594   0.7468 0.5242787
time:posn          6    0.4917   0.0820   0.2360 0.9647710
time:jaw           3    0.7833   0.2611   0.7519 0.5213545
form:surf          3    4.5103   1.5034   4.3293 0.0048340
form:posn          6    1.1842   0.1974   0.5683 0.7557765
form:jaw           3    1.5436   0.5145   1.4817 0.2179425
surf:posn          2    9.2973   4.6487  13.3863 0.0000018
posn:jaw           2    4.5098   2.2549   6.4932 0.0015783
surf:jaw           1  110.0597 110.0597 316.9277 0.0000000
time:form:surf     9    9.9389   1.1043   3.1800 0.0008444
time:form:posn    18    8.5378   0.4743   1.3659 0.1398770
time:form:jaw      9    3.5994   0.3999   1.1516 0.3230920
time:(surf:posn)   6    0.7506   0.1251   0.3602 0.9040757
time:(posn:jaw)    6    0.7161   0.1193   0.3437 0.9137250
time:(surf:jaw)    3    3.7878   1.2626   3.6358 0.0125442
form:(surf:posn)   6    0.1560   0.0260   0.0749 0.9983934
form:(posn:jaw)    6    1.3169   0.2195   0.6320 0.7047109
form:(surf:jaw)    3    0.2511   0.0837   0.2410 0.8677676
surf:posn:jaw      2   16.7945   8.3972  24.1806 0.0000000
Residuals        999  346.9235   0.3473
```

MEAN STAIN INDEX

```
Error: pers
          Df Sum of Sq   Mean Sq   F Value     Pr(F)
form:tim   9  0.838578 0.0931753 0.8899994 0.5556988
Residuals 15  1.570372 0.1046915
Total     24  2.408950 0.100373  2.453146  0.0026077

Error: time %in% pers
          Df Sum of Sq   Mean Sq  F Value     Pr(F)
form       3  0.249228 0.0830759 2.030398 0.1191882
tim        3  0.781411 0.2604703 6.365962 0.0008090
form:tim   9  0.394302 0.0438113 1.070761 0.3973140
Residuals 60  2.454966 0.0409161
```

Analysis of 28 teeth

```
Error: pers
          Df Sum of Sq   Mean Sq  F Value     Pr(F)
time:form  9   7.90565 0.8784058 1.013288 0.4713232
Residuals 15  13.00329 0.8668863

Error: time
     Df Sum of Sq  Mean Sq
time  3  8.520556 2.840185

Error: pers:time
          Df Sum of Sq   Mean Sq  F Value     Pr(F)
form       3   1.67708 0.5590267 1.446703 0.2381912
time:form  9   4.37879 0.4865319 1.259094 0.2779145
Residuals 60  23.18486 0.3864144

Error: Within
                  Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf               1    0.4095  0.40947   3.5767 0.0588855
posn               2   14.3668  7.18340  62.7466 0.0000000
jaw                1   12.8478 12.84780 112.2247 0.0000000
time:surf          3    0.7086  0.23620   2.0632 0.1034693
time:posn          6    1.5549  0.25914   2.2636 0.0355213
time:jaw           3    3.1495  1.04984   9.1703 0.0000055
form:surf          3    0.2840  0.09465   0.8268 0.4791803
form:posn          6    0.6786  0.11311   0.9880 0.4319589
form:jaw           3    1.6246  0.54154   4.7303 0.0027745
surf:posn          2    0.0834  0.04169   0.3642 0.6948737
posn:jaw           2    7.5328  3.76641  32.8994 0.0000000
surf:jaw           1    1.1408  1.14083   9.9651 0.0016431
time:form:surf     9    0.4482  0.04980   0.4350 0.9165175
time:form:posn    18    2.3007  0.12782   1.1165 0.3296866
time:form:jaw      9    6.5879  0.73199   6.3939 0.0000000
time:(surf:posn)   6    2.0866  0.34777   3.0377 0.0059912
time:(posn:jaw)    6    0.4868  0.08113   0.7087 0.6426590
time:(surf:jaw)    3    0.2319  0.07731   0.6753 0.5672381
form:(surf:posn)   6    0.2498  0.04164   0.3637 0.9019995
form:(posn:jaw)    6    0.1288  0.02147   0.1876 0.9803374
form:(surf:jaw)    3    0.0011  0.00036   0.0031 0.9997584
surf:posn:jaw      2    0.6254  0.31271   2.7315 0.0656088
Residuals        999  114.3683  0.11448
```

Analysis of 20 teeth

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
time:form  9  13.76191 1.529101 0.763903 0.6503974
Residuals 15  30.02542 2.001695

Error: time
     Df Sum of Sq Mean Sq
time  3  16.13937 5.37979

Error: pers:time
          Df Sum of Sq   Mean Sq  F Value     Pr(F)
form       3   2.94457 0.9815244 1.235010 0.3049402
time:form  9   6.90019 0.7666878 0.964691 0.4779136
Residuals 60  47.68499 0.7947499

Error: Within
                 Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf              1    0.8225  0.82251   5.0911 0.0242646
posn              2    7.7328  3.86639  23.9319 0.0000000
jaw               1   28.2389 28.23889 174.7910 0.0000000
time:surf         3    2.4554  0.81846   5.0661 0.0017403
time:posn         6    0.5082  0.08470   0.5243 0.7902025
time:jaw          3    4.9343  1.64476  10.1806 0.0000013
form:surf         3    0.8011  0.26705   1.6529 0.1755676
form:posn         6    0.3946  0.06577   0.4071 0.8746141
form:jaw          3    2.4649  0.82162   5.0856 0.0016936
surf:posn         2    0.1667  0.08334   0.5158 0.5971541
posn:jaw          2    3.5081  1.75403  10.8570 0.0000216
surf:jaw          1    2.9751  2.97505  18.4148 0.0000195
time:form:surf    9    1.1798  0.13109   0.8114 0.6057455
time:form:posn   18    1.8420  0.10233   0.6334 0.8753431
time:form:jaw     9   10.5302  1.17002   7.2421 0.0000000
time:(surf:posn)  6    0.7830  0.13050   0.8078 0.5639076
time:(posn:jaw)   6    0.8877  0.14795   0.9158 0.4825881
time:(surf:jaw)   3    0.5945  0.19817   1.2266 0.2987743
form:(surf:posn)  6    0.2063  0.03439   0.2129 0.9728047
form:(posn:jaw)   6    0.0489  0.00814   0.0504 0.9994827
form:(surf:jaw)   3    0.0040  0.00134   0.0083 0.9989617
surf:posn:jaw     2    0.1548  0.07741   0.4792 0.6194417
Residuals       999  161.3965  0.16156
```

TASTE RATING

```
Error: pers
          Df Sum of Sq   Mean Sq   F Value     Pr(F)
form:time  9   4.67388 0.5193200 0.8688038 0.5711047
Residuals 15   8.96612 0.5977413

Error: Within
          Df Sum of Sq  Mean Sq  F Value      Pr(F)
form       3  36.43000 12.14333 20.43509 0.00000000
time       3   4.02452  1.34151  2.25752 0.09087355
form:time  9   9.64111  1.07123  1.80270 0.08639682
Residuals 60  35.65436  0.59424
```

Appendix XI. Analysis of variance (ANOVA) table for TTO 6 week effects on oral health (Part 3)

MEAN PLAQUE INDEX

```
Error: pers
          Df Sum of Sq   Mean Sq  F Value     Pr(F)
form       1   0.73303 0.7330307 2.305769 0.1355931
Residuals 47  14.94185 0.3179116

Error: time %in% pers
          Df Sum of Sq   Mean Sq  F Value     Pr(F)
time       2  0.107205 0.0536024  0.60077 0.5504836
form:time  2  1.961385 0.9806927 10.99141 0.0000513
Residuals 94  8.387011 0.0892235
```

Analysis with 28 teeth
week 0 to 3

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value       Pr(F)
form       1   30.7266 30.72658 9.653838 0.003201855
Residuals 47  149.5933  3.18284
Error: Within
                    Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                 1   21.2445 21.24454 46.52567 0.0000000
posn                 2    2.3087  1.15434  2.52801 0.0808041
jaw                  1    1.5683  1.56826  3.43449 0.0644178
form:surf            1    0.0822  0.08224  0.18010 0.6714630
form:posn            2    0.1058  0.05288  0.11582 0.8906604
surf:posn            2    1.3593  0.67963  1.48839 0.2267017
form:jaw             1    0.6307  0.63067  1.38117 0.2404422
surf:jaw             1    0.3527  0.35265  0.77231 0.3799109
posn:jaw             2    0.4484  0.22421  0.49102 0.6122885
form:surf:posn       2    0.0999  0.04994  0.10937 0.8964220
form:surf:jaw        1    0.8465  0.84655  1.85395 0.1739182
form:posn:jaw        2    0.2309  0.11546  0.25286 0.7766714
surf:posn:jaw        2    0.2725  0.13624  0.29837 0.7421546
form:surf:posn:jaw   2    0.8984  0.44918  0.98372 0.3746180
Residuals          517  236.0724  0.45662
The tables of means are shown in Appendix H.
Positive numbers represent an increase from Week 0 to Week 3.
The effect of formulation is shown in the following table:
          Bu     Li  Mean     sed(form)     = 0.147
     Y 0.021 -0.382  -0.181   sed(surf)     = 0.056
     Z 0.455  0.099   0.277   sed(same row) = 0.079
  Mean 0.233 -0.147   0.043
```

Analysis with 20 teeth
week 0 to 3

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value       Pr(F)
form       1   38.7148 38.71476 10.08287 0.002641832
Residuals 47  180.4639  3.83966
Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1   19.5926 19.59259 36.55987 0.0000000
posn                2    2.0284  1.01422  1.89254 0.1517313
jaw                 1    1.3814  1.38138  2.57766 0.1089926
form:surf           1    0.1927  0.19272  0.35961 0.5489852
form:posn           2    0.7580  0.37902  0.70725 0.4934745
surf:posn           2    2.4264  1.21320  2.26384 0.1049806
form:jaw            1    1.0561  1.05615  1.97077 0.1609661
surf:jaw            1    0.3658  0.36584  0.68265 0.4090568
posn:jaw            2    0.0972  0.04861  0.09071 0.9132984
form:surf:posn      2    0.0565  0.02826  0.05273 0.9486447
form:surf:jaw       1    1.2588  1.25882  2.34896 0.1259782
form:posn:jaw       2    0.4219  0.21094  0.39361 0.6748177
surf:posn:jaw       2    1.1033  0.55163  1.02935 0.3579691
form:surf:posn:jaw  2    1.5396  0.76981  1.43647 0.2387118
Residuals         517  277.0626  0.53590
```

Analysis with 28 teeth
week 0 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value        Pr(F)
form       1  38.44601 38.44601 22.85685 1.757467e-05
Residuals 47  79.05564  1.68203
Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1   23.8344 23.83438 56.51075 0.0000000
posn                2    3.9456  1.97278  4.67743 0.0097002
jaw                 1    0.3734  0.37336  0.88522 0.3472155
form:surf           1    1.5757  1.57574  3.73603 0.0537966
form:posn           2    0.0179  0.00895  0.02123 0.9789938
surf:posn           2    1.9467  0.97334  2.30776 0.1005080
form:jaw            1    0.6198  0.61976  1.46943 0.2259900
surf:jaw            1    1.0029  1.00286  2.37775 0.1236864
posn:jaw            2    0.9519  0.47594  1.12843 0.3243355
form:surf:posn      2    0.0040  0.00199  0.00471 0.9952968
form:surf:jaw       1    0.0078  0.00783  0.01857 0.8916585
form:posn:jaw       2    0.2210  0.11050  0.26200 0.7696139
surf:posn:jaw       2    0.0916  0.04580  0.10859 0.8971184
form:surf:posn:jaw  2    0.7877  0.39387  0.93385 0.3936997
Residuals         517  218.0536  0.42177
```

Analysis with 20 teeth
week 0 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value        Pr(F)
form       1  42.83449 42.83449 21.26279 3.090394e-05
Residuals 47  94.68282  2.01453
Error: Within
                    Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                 1   20.2367 20.23669 43.46265 0.0000000
posn                 2    4.9379  2.46897  5.30265 0.0052528
jaw                  1    0.0737  0.07371  0.15830 0.6908873
form:surf            1    1.8501  1.85014  3.97357 0.0467446
form:posn            2    0.2652  0.13262  0.28484 0.7522539
surf:posn            2    1.0547  0.52736  1.13263 0.3229833
form:jaw             1    0.4203  0.42032  0.90273 0.3424948
surf:jaw             1    0.8045  0.80453  1.72790 0.1892625
posn:jaw             2    1.0216  0.51078  1.09702 0.3346417
form:surf:posn       2    0.0951  0.04753  0.10209 0.9029701
form:surf:jaw        1    0.0510  0.05096  0.10945 0.7409104
form:posn:jaw        2    0.6613  0.33065  0.71015 0.4920496
surf:posn:jaw        2    0.3245  0.16224  0.34844 0.7059534
form:surf:posn:jaw   2    1.1821  0.59104  1.26938 0.2818793
Residuals          517  240.7209  0.46561
```

Analysis with 28 teeth
week 3 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq   F Value     Pr(F)
form       1   0.43208 0.432081 0.3274635 0.5698837
Residuals 47  62.01548 1.319478
Error: Within
                    Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                 1    0.0745 0.074456 0.207792 0.6486947
posn                 2    0.5344 0.267186 0.745661 0.4749299
jaw                  1    0.4112 0.411229 1.147656 0.2845402
form:surf            1    2.3779 2.377930 6.636321 0.0102684
form:posn            2    0.0400 0.019985 0.055774 0.9457584
surf:posn            2    0.1744 0.087211 0.243389 0.7840561
form:jaw             1    0.0000 0.000048 0.000133 0.9908039
surf:jaw             1    0.1661 0.166123 0.463615 0.4962440
posn:jaw             2    0.1286 0.064290 0.179420 0.8358066
form:surf:posn       2    0.0672 0.033602 0.093776 0.9105022
form:surf:jaw        1    1.0172 1.017236 2.838899 0.0926107
form:posn:jaw        2    0.1960 0.098000 0.273497 0.7608243
surf:posn:jaw        2    0.4497 0.224867 0.627559 0.5342997
form:surf:posn:jaw   2    0.6668 0.333386 0.930412 0.3950505
Residuals          517  185.2517 0.358321
```

Analysis with 20 teeth
week 3 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq    F Value     Pr(F)
form       1   0.10413 0.104127 0.06925486 0.7935751
Residuals 47  70.66628 1.503538
Error: Within
                    Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                 1    0.0052 0.005208 0.012410 0.9113425
posn                 2    0.6574 0.328692 0.783176 0.4574936
jaw                  1    0.8169 0.816905 1.946446 0.1635694
form:surf            1    3.2371 3.237099 7.713058 0.0056813
form:posn            2    0.2137 0.106856 0.254606 0.7753187
surf:posn            2    0.3969 0.198448 0.472844 0.6234967
form:jaw             1    0.1439 0.143920 0.342920 0.5584040
surf:jaw             1    0.0853 0.085329 0.203315 0.6522476
posn:jaw             2    0.6974 0.348722 0.830903 0.4362368
form:surf:posn       2    0.0193 0.009646 0.022984 0.9772795
form:surf:jaw        1    0.8032 0.803228 1.913856 0.1671326
form:posn:jaw        2    0.3714 0.185715 0.442503 0.6426692
surf:posn:jaw        2    1.1359 0.567968 1.353302 0.2592995
form:surf:posn:jaw   2    0.5628 0.281422 0.670547 0.5118731
Residuals          517  216.9801 0.419691
```

MEAN STAIN INDEX

```
Error: pers
          Df Sum of Sq   Mean Sq  F Value     Pr(F)
form       1   0.91105 0.9110535 2.451308 0.1241359
Residuals 47  17.46803 0.3716602

Error: time %in% pers
          Df Sum of Sq   Mean Sq  F Value       Pr(F)
time       2  0.621106 0.3105529 7.054700 0.001397741
form:time  2  0.560002 0.2800009 6.360662 0.002565679
Residuals 94  4.137947 0.0440207
```

Analysis with 28 teeth
week 0 to 3

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       1   3.97519 3.975193 5.528901 0.0229491
Residuals 47  33.79226 0.718984
Error: Within
                    Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                 1    2.5165 2.516470 9.315169 0.0023895
posn                 2    0.4460 0.222976 0.825387 0.4386420
jaw                  1    1.9583 1.958284 7.248943 0.0073244
form:surf            1    1.6613 1.661332 6.149719 0.0134607
form:posn            2    0.7130 0.356506 1.319671 0.2681219
surf:posn            2    0.1908 0.095382 0.353073 0.7026954
form:jaw             1    0.2012 0.201184 0.744720 0.3885531
surf:jaw             1    1.4435 1.443454 5.343207 0.0211954
posn:jaw             2    0.6891 0.344537 1.275366 0.2802060
form:surf:posn       2    1.0005 0.500227 1.851680 0.1580127
form:surf:jaw        1    0.1411 0.141061 0.522161 0.4702480
form:posn:jaw        2    0.2096 0.104775 0.387845 0.6787150
surf:posn:jaw        2    0.8980 0.449010 1.662091 0.1907541
form:surf:posn:jaw   2    0.4327 0.216344 0.800837 0.4495092
Residuals          517  139.6663 0.270148
```

Analysis with 20 teeth
week 0 to 3

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value      Pr(F)
form       1   6.86337 6.863365 4.188106 0.04632454
Residuals 47  77.02245 1.638776
Error: Within
                    Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                 1    3.9730 3.972978 10.71667 0.0011328
posn                 2    0.2223 0.111158  0.29984 0.7410675
jaw                  1    3.3426 3.342593  9.01627 0.0028054
form:surf            1    3.3712 3.371236  9.09353 0.0026913
form:posn            2    0.0181 0.009074  0.02448 0.9758219
surf:posn            2    0.1339 0.066941  0.18057 0.8348510
form:jaw             1    0.3301 0.330078  0.89035 0.3458235
surf:jaw             1    4.1671 4.167092 11.24027 0.0008591
posn:jaw             2    0.9054 0.452712  1.22114 0.2957431
form:surf:posn       2    0.2170 0.108523  0.29273 0.7463476
form:surf:jaw        1    0.0912 0.091242  0.24612 0.6200339
form:posn:jaw        2    0.4062 0.203084  0.54780 0.5785576
surf:posn:jaw        2    0.3200 0.160006  0.43160 0.6497045
form:surf:posn:jaw   2    0.9553 0.477657  1.28843 0.2765877
Residuals          517  191.6668 0.370729
```

Analysis with 28 teeth
week 0 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value       Pr(F)
form       1  12.45837 12.45837 10.95654 0.001796871
Residuals 47  53.44237  1.13707
Error: Within
                    Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                 1    3.8452 3.845242 16.40963 0.0000589
posn                 2    1.1722 0.586112  2.50124 0.0829750
jaw                  1    3.8130 3.812963 16.27188 0.0000632
form:surf            1    0.0269 0.026945  0.11499 0.7346721
form:posn            2    0.0991 0.049539  0.21141 0.8095118
surf:posn            2    1.1287 0.564347  2.40836 0.0909716
form:jaw             1    1.3836 1.383603  5.90455 0.0154412
surf:jaw             1    1.8577 1.857657  7.92758 0.0050545
posn:jaw             2    0.5303 0.265146  1.13151 0.3233418
form:surf:posn       2    0.2644 0.132184  0.56410 0.5692232
form:surf:jaw        1    0.7344 0.734357  3.13388 0.0772704
form:posn:jaw        2    0.2664 0.133200  0.56843 0.5667662
surf:posn:jaw        2    0.0295 0.014772  0.06304 0.9389141
form:surf:posn:jaw   2    0.0920 0.046012  0.19636 0.8217791
Residuals          517  121.1478 0.234328
```

Analysis with 20 teeth
week 0 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value       Pr(F)
form       1  18.35290 18.35290 9.481447 0.003461009
Residuals 47  90.97625  1.93566
Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1    7.0935 7.093549 18.40515 0.0000213
posn                2    0.0248 0.012413  0.03221 0.9683089
jaw                 1    5.7047 5.704660 14.80149 0.0001344
form:surf           1    0.0163 0.016336  0.04239 0.8369670
form:posn           2    1.1663 0.583167  1.51310 0.2211990
surf:posn           2    0.4304 0.215219  0.55841 0.5724608
form:jaw            1    3.1803 3.180302  8.25171 0.0042386
surf:jaw            1    2.8705 2.870477  7.44783 0.0065677
posn:jaw            2    0.8865 0.443252  1.15007 0.3174218
form:surf:posn      2    0.2727 0.136356  0.35379 0.7021891
form:surf:jaw       1    0.4771 0.477055  1.23778 0.2664159
form:posn:jaw       2    0.0664 0.033215  0.08618 0.9174406
surf:posn:jaw       2    0.4248 0.212384  0.55106 0.5766773
form:surf:posn:jaw  2    0.6171 0.308527  0.80051 0.4496537
Residuals         517  199.2576 0.385411
```

Analysis with 28 teeth
week 3 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value      Pr(F)
form       1   2.35884 2.358844 3.010098 0.08930127
Residuals 47  36.83124 0.783643
Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1    0.1403 0.140318 0.528670 0.4674959
posn                2    0.2176 0.108784 0.409862 0.6639571
jaw                 1    0.3061 0.306134 1.153408 0.2833378
form:surf           1    1.2651 1.265123 4.766546 0.0294669
form:posn           2    0.3208 0.160397 0.604319 0.5468319
surf:posn           2    0.4011 0.200550 0.755604 0.4702446
form:jaw            1    0.5296 0.529592 1.995320 0.1583874
surf:jaw            1    0.0261 0.026089 0.098294 0.7540130
posn:jaw            2    0.3732 0.186591 0.703009 0.4955660
form:surf:posn      2    0.4278 0.213901 0.805904 0.4472441
form:surf:jaw       1    0.2317 0.231713 0.873014 0.3505583
form:posn:jaw       2    0.3762 0.188111 0.708739 0.4927424
surf:posn:jaw       2    0.8898 0.444881 1.676160 0.1881062
form:surf:posn:jaw  2    0.3495 0.174743 0.658373 0.5181266
Residuals         517  137.2206 0.265417
```

Analysis with 20 teeth
week 3 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       1   2.76965 2.769649 2.444766 0.1246264
Residuals 47  53.24580 1.132889
Error: Within
                    Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                 1    0.4491 0.449086 1.172942 0.2793025
posn                 2    0.3390 0.169513 0.442741 0.6425167
jaw                  1    0.3138 0.313787 0.819563 0.3657296
form:surf            1    2.9182 2.918221 7.621934 0.0059711
form:posn            2    1.2823 0.641147 1.674574 0.1884027
surf:posn            2    0.2888 0.144381 0.377100 0.6860363
form:jaw             1    1.4612 1.461236 3.816518 0.0512885
surf:jaw             1    0.1205 0.120477 0.314666 0.5750739
posn:jaw             2    0.1505 0.075267 0.196585 0.8215926
form:surf:posn       2    0.1450 0.072510 0.189384 0.8275260
form:surf:jaw        1    0.1510 0.151032 0.394471 0.5302357
form:posn:jaw        2    0.1746 0.087303 0.228020 0.7961880
surf:posn:jaw        2    0.7163 0.358147 0.935423 0.3930830
form:surf:posn:jaw   2    0.1556 0.077806 0.203218 0.8161658
Residuals          517  197.9445 0.382871
```

MEAN GINGIVAL INDEX

```
Error: pers
          Df Sum of Sq   Mean Sq   F Value     Pr(F)
form       1  0.037349 0.0373489 0.1937661 0.6618183
Residuals 47  9.059363 0.1927524

Error: time %in% pers
          Df Sum of Sq   Mean Sq  F Value     Pr(F)
time       2  0.246433 0.1232166 3.516678 0.0336639
form:time  2  0.038830 0.0194149 0.554114 0.5764466
Residuals 94  3.293551 0.0350378
```

Analysis with 28 teeth
week 0 to 3

```
Error: pers
          Df Sum of Sq  Mean Sq   F Value     Pr(F)
form       1   0.20778 0.207782 0.2026722 0.6546423
Residuals 47  48.18501 1.025213
Error: Within
                    Df Sum of Sq   Mean Sq  F Value     Pr(F)
surf                 1    0.7370 0.7369619 1.843954 0.1750806
posn                 2    0.1988 0.0994232 0.248767 0.7798549
jaw                  1    0.1393 0.1392900 0.348518 0.5552114
form:surf            1    0.0972 0.0972020 0.243209 0.6221071
form:posn            2    0.0237 0.0118471 0.029643 0.9707941
surf:posn            2    0.3164 0.1581779 0.395777 0.6733604
form:jaw             1    0.3070 0.3069610 0.768048 0.3812287
surf:jaw             1    0.9946 0.9946150 2.488628 0.1152833
posn:jaw             2    0.1093 0.0546528 0.136747 0.8722225
form:surf:posn       2    0.1229 0.0614663 0.153795 0.8574869
form:surf:jaw        1    0.8128 0.8127619 2.033613 0.1544580
form:posn:jaw        2    0.6717 0.3358690 0.840379 0.4321357
surf:posn:jaw        2    1.6439 0.8219676 2.056647 0.1289270
form:surf:posn:jaw   2    0.6644 0.3321975 0.831192 0.4361110
Residuals          517  206.6263 0.3996639
```

Analysis with 20 teeth

week 0 to 3

```
Error: pers
          Df Sum of Sq   Mean Sq   F Value     Pr(F)
form       1   0.36701 0.3670069 0.3913667 0.534608
Residuals 47  44.07458 0.9377570
Error: Within
                    Df Sum of Sq   Mean Sq  F Value     Pr(F)
surf                 1    0.0043 0.0042635 0.010528 0.9183173
posn                 2    0.1672 0.0835813 0.206380 0.8135908
jaw                  1    0.0113 0.0113497 0.028025 0.8671161
form:surf            1    0.1446 0.1445598 0.356949 0.5504673
form:posn            2    0.0322 0.0161216 0.039808 0.9609772
surf:posn            2    0.7251 0.3625402 0.895189 0.4091628
form:jaw             1    0.1439 0.1439204 0.355370 0.5513498
surf:jaw             1    0.1672 0.1672454 0.412964 0.5207536
posn:jaw             2    0.5105 0.2552556 0.630280 0.5328512
form:surf:posn       2    0.2184 0.1092230 0.269695 0.7637200
form:surf:jaw        1    0.7067 0.7067284 1.745063 0.1870830
form:posn:jaw        2    0.5358 0.2678849 0.661465 0.5165311
surf:posn:jaw        2    1.8532 0.9265991 2.287970 0.1024988
form:surf:posn:jaw   2    1.0845 0.5422358 1.338895 0.2630425
Residuals          517  209.3785 0.4049874
```

Analysis with 28 teeth

week 0 to 6

```
(iii) Gingival
There is a surface by jaw interaction, but it is not particularly
strong.  Since there
is nothing involving formulation, this is not pursued here.
Error: pers
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       1   1.05494 1.054936 1.202616 0.2783867
Residuals 47  41.22844 0.877201
Error: Within
                    Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                 1    0.7656 0.765556 2.167934 0.1415239
posn                 2    0.1795 0.089754 0.254169 0.7756577
jaw                  1    1.1630 1.162963 3.293329 0.0701412
form:surf            1    0.0060 0.006033 0.017083 0.8960612
form:posn            2    0.4029 0.201436 0.570435 0.5656347
surf:posn            2    0.0338 0.016917 0.047905 0.9532285
form:jaw             1    0.9013 0.901313 2.552376 0.1107395
surf:jaw             1    2.3640 2.364025 6.694548 0.0099426
posn:jaw             2    1.0719 0.535937 1.517689 0.2201930
form:surf:posn       2    0.3173 0.158660 0.449301 0.6383227
form:surf:jaw        1    0.0082 0.008153 0.023089 0.8792845
form:posn:jaw        2    0.1062 0.053101 0.150374 0.8604236
surf:posn:jaw        2    1.4742 0.737098 2.087345 0.1250598
form:surf:posn:jaw   2    0.0459 0.022956 0.065007 0.9370682
Residuals          517  182.5667 0.353127
```

Analysis with 20 teeth
week 0 to 6

```
Error: pers
          Df Sum of Sq   Mean Sq  F Value    Pr(F)
form       1   0.76718 0.7671770 1.051886 0.310323
Residuals 47  34.27874 0.7293349
Error: Within
                    Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                 1    1.3333 1.333333 3.102658 0.0787556
posn                 2    0.5548 0.277400 0.645508 0.5248184
jaw                  1    2.3916 2.391582 5.565194 0.0186919
form:surf            1    0.0851 0.085078 0.197976 0.6565456
form:posn            2    0.1900 0.095005 0.221075 0.8017321
surf:posn            2    0.6769 0.338435 0.787537 0.4555091
form:jaw             1    0.9720 0.971961 2.261747 0.1332150
surf:jaw             1    1.4468 1.446759 3.366599 0.0671053
posn:jaw             2    0.3761 0.188067 0.437630 0.6458033
form:surf:posn       2    1.2183 0.609143 1.417471 0.2432662
form:surf:jaw        1    0.0438 0.043759 0.101826 0.7497780
form:posn:jaw        2    0.2627 0.131332 0.305609 0.7368074
surf:posn:jaw        2    1.1084 0.554186 1.289586 0.2762691
form:surf:posn:jaw   2    0.0310 0.015511 0.036093 0.9645529
Residuals          517  222.1751 0.429739
```

Analysis with 28 teeth
week 3 to 6

```
Error: pers
          Df Sum of Sq   Mean Sq   F Value     Pr(F)
form       1   0.32635 0.3263486 0.3860618 0.5373789
Residuals 47  39.73039 0.8453274
Error: Within
                    Df Sum of Sq   Mean Sq  F Value     Pr(F)
surf                 1    0.0003 0.0002721 0.000694 0.9789920
posn                 2    0.0856 0.0428009 0.109174 0.8965951
jaw                  1    0.4973 0.4972959 1.268471 0.2605761
form:surf            1    0.1517 0.1516650 0.386858 0.5342300
form:posn            2    0.5540 0.2770135 0.706589 0.4938000
surf:posn            2    0.5269 0.2634453 0.671980 0.5111419
form:jaw             1    0.1563 0.1562893 0.398653 0.5280648
surf:jaw             1    0.2919 0.2918537 0.744442 0.3886416
posn:jaw             2    1.2629 0.6314300 1.610612 0.2007659
form:surf:posn       2    0.7896 0.3947973 1.007024 0.3660197
form:surf:jaw        1    0.6581 0.6581049 1.678653 0.1956817
form:posn:jaw        2    0.2512 0.1255997 0.320372 0.7260231
surf:posn:jaw        2    0.9595 0.4797293 1.223664 0.2950010
form:surf:posn:jaw   2    0.7230 0.3615048 0.922104 0.3983345
Residuals          517  202.6865 0.3920434
```

Analysis with 20 teeth
week 3 to 6

```
Error: pers
          Df Sum of Sq   Mean Sq    F Value     Pr(F)
form       1   0.07294 0.0729408 0.08559649 0.7711395
Residuals 47  40.05090 0.8521468
Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1    1.4884 1.488390 3.624560 0.0574876
posn                2    1.1057 0.552875 1.346372 0.2610932
jaw                 1    2.0734 2.073425 5.049247 0.0250577
form:surf           1    0.0078 0.007837 0.019086 0.8901751
form:posn           2    0.2528 0.126397 0.307805 0.7351932
surf:posn           2    1.0666 0.533317 1.298744 0.2737630
form:jaw            1    0.3679 0.367857 0.895814 0.3443488
surf:jaw            1    0.6302 0.630208 1.534697 0.2159711
posn:jaw            2    0.0315 0.015743 0.038338 0.9623904
form:surf:posn      2    0.5009 0.250469 0.609947 0.5437702
form:surf:jaw       1    0.3988 0.398774 0.971103 0.3248662
form:posn:jaw       2    0.0516 0.025817 0.062869 0.9390738
surf:posn:jaw       2    0.7783 0.389137 0.947634 0.3883293
form:surf:posn:jaw  2    1.4802 0.740123 1.802364 0.1659432
Residuals         517  212.3011 0.410640
```

MEAN BLEEDING INDEX

```
Error: pers
Df Sum of Sq  Mean Sq   F Value     Pr(F)
form       1   0.95460 0.954600 0.7992301 0.3758789
Residuals 47  56.13675 1.194399

Error: time %in% pers
          Df Sum of Sq   Mean Sq  F Value     Pr(F)
time       2   0.02182 0.0109122 0.071315 0.9312193
form:time  2   0.37158 0.1857876 1.214178 0.3015681
Residuals 94  14.38342 0.1530151
```

Analysis with 28 teeth
week 0 to 3

```
(iv) Bleeding
The analysis for bleeding likewise shows no significant effects.
Error: pers
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       1    5.1663 5.166327 1.374954 0.2468716
Residuals 47  176.6003 3.757454
Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1    1.3033 1.303345 1.031018 0.3103945
posn                2    1.4077 0.703841 0.556777 0.5733960
jaw                 1    0.0003 0.000295 0.000234 0.9878124
form:surf           1    2.5506 2.550572 2.017645 0.1560829
form:posn           2    0.2751 0.137552 0.108811 0.8969203
surf:posn           2    1.8224 0.911211 0.720818 0.4868422
form:jaw            1    0.0485 0.048543 0.038400 0.8447182
surf:jaw            1    0.4454 0.445409 0.352343 0.5530494
posn:jaw            2    0.9259 0.462951 0.366220 0.6935299
form:surf:posn      2    2.9062 1.453112 1.149493 0.3176057
form:surf:jaw       1    0.5769 0.576874 0.456340 0.4996416
form:posn:jaw       2    2.6236 1.311805 1.037710 0.3550016
surf:posn:jaw       2    0.1627 0.081336 0.064341 0.9376928
form:surf:posn:jaw  2    0.9223 0.461130 0.364779 0.6945284
Residuals         517  653.5571 1.264134
```

Analysis with 20 teeth
week 0 to 3

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       1    5.6846 5.684603 1.544969 0.2200449
Residuals 47  172.9331 3.679428

Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1    1.7506 1.750579 1.226184 0.2686654
posn                2    1.9311 0.965573 0.676331 0.5089287
jaw                 1    0.3373 0.337313 0.236269 0.6271196
form:surf           1    1.3113 1.311300 0.918493 0.3383188
form:posn           2    0.3289 0.164466 0.115199 0.8912116
surf:posn           2    0.4365 0.218266 0.152883 0.8582688
form:jaw            1    0.0040 0.004021 0.002817 0.9576936
surf:jaw            1    0.1508 0.150805 0.105631 0.7453057
posn:jaw            2    1.2273 0.613674 0.429845 0.6508424
form:surf:posn      2    6.7951 3.397560 2.379803 0.0935822
form:surf:jaw       1    0.8658 0.865761 0.606418 0.4364961
form:posn:jaw       2    1.7778 0.888899 0.622625 0.5369360
surf:posn:jaw       2    1.3501 0.675040 0.472828 0.6235063
form:surf:posn:jaw  2    2.4948 1.247417 0.873747 0.4180001
Residuals         517  738.1024 1.427664
```

Analysis with 28 teeth
week 0 to 6

(iv) Bleeding
The analysis shows that there may be an interaction of formulation and position.

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value      Pr(F)
form       1   10.6297 10.62971 3.370327 0.07271131
Residuals 47  148.2338  3.15391
Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1    7.4156 7.415648 5.745902 0.0168811
posn                2    5.3751 2.687551 2.082408 0.1256738
jaw                 1    1.6308 1.630841 1.263632 0.2614863
form:surf           1    3.4278 3.427835 2.656006 0.1037686
form:posn           2   11.4375 5.718725 4.431067 0.0123572
surf:posn           2    0.8195 0.409745 0.317485 0.7281194
form:jaw            1    0.0140 0.013968 0.010823 0.9171823
surf:jaw            1    0.0272 0.027211 0.021084 0.8846071
posn:jaw            2    0.0125 0.006273 0.004861 0.9951512
form:surf:posn      2    0.2030 0.101507 0.078651 0.9243737
form:surf:jaw       1    1.2725 1.272519 0.985992 0.3211885
form:posn:jaw       2    0.8165 0.408272 0.316343 0.7289503
surf:posn:jaw       2    5.9269 2.963471 2.296200 0.1016660
form:surf:posn:jaw  2    3.7484 1.874187 1.452185 0.2350114
Residuals         517  667.2390 1.290598
```

Analysis with 20 teeth
week 0 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq   F Value     Pr(F)
form       1    2.5050 2.504979 0.7616459 0.3872509
Residuals 47  154.5784 3.288903
Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1    7.8062 7.806229 5.300904 0.0217107
posn                2    7.4095 3.704755 2.515754 0.0817908
jaw                 1    1.0559 1.055851 0.716987 0.3975258
form:surf           1    6.4422 6.442198 4.374644 0.0369648
form:posn           2    2.5159 1.257966 0.854235 0.4262083
surf:posn           2    2.7366 1.368304 0.929161 0.3955431
form:jaw            1    0.7569 0.756889 0.513974 0.4737474
surf:jaw            1    0.7322 0.732249 0.497242 0.4810322
posn:jaw            2    0.1797 0.089864 0.061023 0.9408080
form:surf:posn      2    0.3375 0.168769 0.114605 0.8917414
form:surf:jaw       1    0.8105 0.810537 0.550404 0.4584895
form:posn:jaw       2    7.1217 3.560827 2.418018 0.0901050
surf:posn:jaw       2    4.9248 2.462408 1.672125 0.1888618
form:surf:posn:jaw  2    6.1701 3.085043 2.094932 0.1241222
Residuals         517  761.3457 1.472622
```

Analysis with 28 teeth
week 3 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq   F Value     Pr(F)
form       1    0.9749 0.974896 0.2437238 0.6238286
Residuals 47  188.0000 4.000001
Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1    2.5012 2.501229 2.251078 0.1341318
posn                2    8.4806 4.240316 3.816236 0.0226331
jaw                 1    1.6750 1.675023 1.507502 0.2200794
form:surf           1    0.0647 0.064714 0.058242 0.8093919
form:posn           2   11.0166 5.508321 4.957427 0.0073689
surf:posn           2    3.7714 1.885682 1.697092 0.1842346
form:jaw            1    0.0104 0.010432 0.009389 0.9228468
surf:jaw            1    0.2524 0.252438 0.227191 0.6338156
posn:jaw            2    0.7320 0.365995 0.329392 0.7195121
form:surf:posn      2    4.4993 2.249627 2.024639 0.1330871
form:surf:jaw       1    0.1358 0.135820 0.122236 0.7267640
form:posn:jaw       2    2.3504 1.175189 1.057657 0.3480187
surf:posn:jaw       2    4.1963 2.098130 1.888293 0.1523721
form:surf:posn:jaw  2    3.4444 1.722216 1.549975 0.2132379
Residuals         517  574.4517 1.111125
```

Analysis with 20 teeth
week 3 to 6

```
Error: pers
          Df Sum of Sq  Mean Sq  F Value     Pr(F)
form       1    0.6424 0.642447 0.149248 0.7009985
Residuals 47  202.3144 4.304561
Error: Within
                   Df Sum of Sq  Mean Sq  F Value     Pr(F)
surf                1    2.1635 2.163454 1.678809 0.1956610
posn                2    6.2249 3.112434 2.415203 0.0903567
jaw                 1    2.5867 2.586735 2.007269 0.1571492
form:surf           1    1.9405 1.940528 1.505822 0.2203363
form:posn           2    1.2491 0.624535 0.484630 0.6162047
surf:posn           2    2.5503 1.275132 0.989484 0.3724714
form:jaw            1    0.8713 0.871252 0.676079 0.4113192
surf:jaw            1    0.2184 0.218443 0.169509 0.6807196
posn:jaw            2    1.2937 0.646825 0.501927 0.6056576
form:surf:posn      2    4.5549 2.277473 1.767286 0.1718262
form:surf:jaw       1    0.0009 0.000910 0.000706 0.9788116
form:posn:jaw       2    2.6567 1.328349 1.030780 0.3574607
surf:posn:jaw       2   11.4063 5.703137 4.425551 0.0124244
form:surf:posn:jaw  2    4.1965 2.098253 1.628214 0.1972847
Residuals         517  666.2496 1.288684
```

APPENDIX XII

PROJECT NO: H/.....

Date Received: .....

THE UNIVERSITY OF ADELAIDE
HUMAN RESEARCH ETHICS COMMITTEE

Applications will be considered in terms of the University's guidelines on the ethics of human research, based on the NH&MRC Statement of Human Experimentation (refer application Information Kit which includes list of headings applying to all applications). Submit the application plus 9 duplicate copies to the Secretary, Human Research Ethics Committee, Secretariat, Office of the Vice-Chancellor.

COVER SHEET FOR ATTACHMENT TO APPLICATION FOR ETHICAL APPROVAL OF A RESEARCH PROJECT INVOLVING HUMAN SUBJECTS

APPLICANT: **Surname:** CHONG / HIRSCH **Initials:** A.Y.L. / R.S. **Title:** DR / DR

DEPARTMENT: DENTISTRY **Tel:** [redacted]

OTHERS INVOLVED:

(Please indicate if a higher degree candidate)

PROJECT TITLE: THE EFFECTS OF A CHLORHEXIDINE CONTAINING TOOTHPASTE ON DENTAL PLAQUE FORMATION AND ON CHRONIC GINGIVITIS.

SOURCE OF FUNDING: HAMILTON LABORATORIES PTY LTD

DATE PROJECT TO BEGIN: June 1998

ESTIMATED DURATION OF PROJECT: 12-18 MONTHS

LOCATION OF RESEARCH: Adelaide Dental Hospital & Department of Dentistry, University of Adelaide

BRIEF DESCRIPTION OF THE PLAN/DESIGN OF PROJECT (in lay terms):

There are 2 parts to this study which involves 120 subjects.

Part 1 is a double blind cross over 4 day study involving 30 subjects (in 4 groups), in whom the effect of a chlorhexidine toothpaste on plaque growth will be compared to a standard toothpaste, and a chlorhexidine mouthwash. The subjects will be monitored for amount of plaque accumulation on the 4th day.

Part 2 is a double blind 12 week study involving 90 subjects, in whom the longer term effects of a chlorhexidine toothpaste on chronic gingivitis will be assessed.

BRIEF DESCRIPTION OF THE AIMS OF PROJECT (in lay terms):

Mouthwashes containing the antibacterial agent chlorhexidine (CHX) are commercially available and have been proven to be safe and effective as anti-plaque and anti-gingivitis agents[1,3,9,10,12,13,14]. However, they taste bitter and their prolonged use can cause staining of teeth and restorations. Previous formulations of chlorhexidine toothpastes have not been stable, had poor taste and have had disappointing effects in reducing plaque growth. A new formulation has shown encouraging anti-plaque effects in a pilot study[5].

The aim of this study is to test this product in human volunteers for their effects on the amount of plaque growth over 4 days and their longer term effects on existing chronic gingivitis over 12 weeks.

ETHICAL IMPLICATIONS OF PROJECT:

The long term use of CHX dental care products has no deleterious effects other than minor reversible staining[2,6] of teeth. CHX mouthwashes are available as over-the-counter items in pharmacies and supermarkets.

In Part 1 of the study, the cessation of toothbrushing for 4 days will result in plaque formation, which is readily removed by professional dental prophylaxis.

Part 2 will assess the effects of a chlorhexidine containing toothpaste on subjects with existing chronic gingivitis. A further exposure of 12 weeks to chronic gingivitis is not considered to adversely affect their periodontal status. At the end of the study, the subjects will receive treatment for their gingivitis, which they would probably not have received if they had not participated.

DRUGS:	Will drugs be administered to subjects? If YES - give name of drug(s):	Chlorhexidine dicluconate
	Will this project be conducted under the Clinical Trials Notification (CTN) Scheme?	YES
	Is Commonwealth Department of Health permission required?	NO
	Has Commonwealth Department of Health permission been obtained?	NO
	Is the administration for therapeutic purposes?	YES (anti-plaque)
	Dosage: 0.75% Method of administration: in toothpaste, not for ingestion	

SUBJECTS:

Source: University students

Age range: 18-40 years old

Selection criteria: Dentate, healthy

Exclusion criteria: Smokers, on medication (prolonged antibiotic therapy, steroids), diabetic, subjects requiring antibiotic cover, patients with pacemakers, hepatic disease, kidney disease, pregnant, lactating females, subjects with periodontitis.

SIGNATURE OF ALL INVESTIGATORS NAMED IN THE PROTOCOL:

Dr A Chong _____

Dr R Hirsch _____

Date:

THE UNIVERSITY OF ADELAIDE HUMAN RESEARCH ETHICS COMMITTEE

PROJECT NO:

APPROVED BY THE COMMITTEE AT THE MEETING HELD ON:

SUBJECT TO:

FOR THE PERIOD UNTIL:

Signed: ..... Date:

Convener

THE UNIVERSITY OF ADELAIDE
HUMAN RESEARCH ETHICS COMMITTEE

1. TITLE

The effects of a chlorhexidine containing toothpaste on dental plaque formation and on chronic gingivitis.

2. INVESTIGATORS & QUALIFICATIONS

Dr A.Y.L.Chong, B.D.S. Postgraduate student in Masters in Periodontics, Department of Dentistry, The University of Adelaide.

Dr R.S. Hirsch, M.D.S., Ph.D., Specialist Periodontist, Senior Lecturer, Department of Dentistry, The University of Adelaide.

3. PURPOSE OF STUDY

Mouthwashes containing the antibacterial agent chlorhexidine (CHX) are commercially available and have been proven to be safe and effective as anti-plaque and anti-gingivitis agents. However, they taste bitter and their prolonged use can cause staining of teeth and restorations. Previous formulations of chlorhexidine toothpastes have not been stable and have had disappointing effects in reducing plaque growth. A new formulation has shown encouraging anti-plaque effects in a pilot study. The aim of this study is to test this product in volunteers for its effects on the amount of plaque growth and on chronic gingivitis.

4. BACKGROUND

Dental plaque

Dental plaque is a bacterial aggregation on teeth and other oral structures[4]. The first line of defence of the gum against dental plaque includes the rapid and constant shedding of epithelial cells, the flushing action of crevicular fluid and its immunoglobulin content, and the passage and activity of neutrophils into the gingival crevice.

If plaque is present around the gingival margins of teeth, gingivitis will gradually develop. Gingivitis is the second line of defence of the gingival tissue to dental plaque bacteria and their products. Gingivitis can be reversed with a professional dental scale and clean, comprising of the mechanical removal of plaque, calculus and stain with scalers; and a dental prophylaxis. A dental prophylaxis involves the polishing of teeth with a rubber cup and a fluoride-containing paste.

Chlorhexidine

Chlorhexidine's anti-plaque and other oral effects have been tested for the last 25 years in numerous studies[3,10,12,13,14]. Previous efforts to incorporate chlorhexidine into toothpaste have encountered problems with its stability and lack of effectiveness as an anti-plaque agent. Many toothpaste ingredients, mainly the anionic detergents will inactivate chlorhexidine[7]. Problems of

formulation, together with the side-effects of tooth staining and disturbance of taste have limited its use when delivered in this way.

Hamilton Laboratories have developed a chlorhexidine-containing toothpaste with encouraging results in a small pilot study[5]. In this case, the short term study showed that the anti-plaque effects were significant. Hence, the present study aims to further evaluate the anti-plaque effects, staining levels and ability to inhibit the development of gingivitis.

5. PRELIMINARY STUDY (if any)

A pilot study[5] showed promising anti-plaque effects of the newly formulated chlorhexidine toothpaste (University of Adelaide Human Ethics Committeeproject number: H/ 36 / 92).

6. SUBJECTS

These will be (healthy, dentate volunteers aged from 18-40 years old. There will be 30 volunteers in the first part of this study. The second part of the study (12 weeks) will also involve 90 volunteers).

7. EXCLUSION CRITERIA (specific)

Smokers, people on medication (prolonged antibiotic therapy, steroids), diabetic, people requiring antibiotic cover, people with pacemakers, hepatic disease, kidney disease, pregnant, lactating females, people with periodontitis.

8. PLAN & DESIGN

Part 1: Effect of a chlorhexidine-containing toothpaste on dental plaque growth

30 healthy volunteers between 18-40 years of age will participate in this study. This is a double-blind cross-over study of 3 preparations and a single blind study of one preparation. Each subject will go through the same experimental procedure 4 times (using a different preparation each time).

Subjects will meet the following inclusion criteria of a clear medical history and have at least 20 natural teeth.
Medical histories will be taken to exclude the conditions described in Item 7.

At the first visit, the subjects will be given the information sheet to read and asked to sign the written consent form. Subjects will then receive a dental examination and a scale and clean, followed by a dental prophylaxis to remove all plaque.

The subjects will be issued with a coded container with one of the following formulations:

	Preparation
1	Chlorhexidine toothpaste slurry
2	Non chlorhexidine toothpaste slurry
3	Colgate Total toothpaste slurry

All subjects will use the following as the final rinse in the study

4	0.12% chlorhexidine mouthwash

The subjects will be required to rinse twice daily with 10ml of the preparation for 1 minute for 4 days. Subjects will be requested not to use any mechanical form of oral hygiene during the 4 days of the study.

On Day 4, the subjects will return to the clinic to have plaque and staining levels scored. Disclosing solution (a vegetable based dye) will be applied to the teeth to make the plaque visible and their teeth will be photographed. Subjects will be questioned about the acceptability of taste of the products used and any adverse reactions.

Day 0	4
Scale & clean	Plaque score
Prophylaxis	Staining score
	Prophylaxis

Following a 'wash-out' period of 1 week, to negate any effects of active ingredients in the toothpaste slurries, each subject will return to repeat the procedure with one of the other preparations as follows:

Day 0	4
Prophylaxis	Plaque score
	Staining score
	Prophylaxis

This process will be repeated until all the Preparations have been tested. All subjects will use Preparation 4 as the final rinse in the study.

Part 2: Effects of chlorhexidine containing toothpaste on chronic gingivitis

90 (3 groups, 30 subjects per group) healthy university student volunteers between 18-45 years of age will participate in this study. They will meet the following inclusion criteria of a clear medical history and have at least 20 natural teeth and have chronic gingivitis. Medical histories will be taken to exclude the conditions described in item 7.

At the first visit (Day 0), the subjects will be given the information sheet to read and asked to sign the written consent form. The subjects will receive a dental examination to score their plaque levels, extrinsic stain level and gingival health.

Following the baseline examination, subjects will be categorised according to their age, plaque and gingivitis scores[8,11]. Gingivitis will be scored by inspection of the gingiva and recording the bleeding on probing (BOP) the earliest clinical sign of the development of gingivitis. A constant force periodontal probe will be used to elicit bleeding on probing of the gingival tissues. The subjects will then be distributed amongst the following treatment groups, so that each group has similar oral health and age characteristics.

The subjects will be issued with one of the following preparations:

Group	*Preparation*
1	Chlorhexidine toothpaste
2	Non chlorhexidine toothpaste
3	Colgate Total® toothpaste

Subjects will be asked to brush as they normally would twice daily, replacing their usual toothpaste with one of the above preparations which will be supplied in a coded tube. Subjects will be requested not to use any mouthwashes during the trial.

On Weeks 4 & 8, the subjects will return to the clinic to have plaque level, extrinsic stain level and gingivitis scored. Disclosing solution (a vegetable based dye) will be applied to the teeth to make the plaque visible and their teeth will be photographed. The subjects will be issued with a new toothbrush.

The same parameters will be measured on Week 12, when the subjects will also receive a scale and clean (to return the gingival tissue to health) and a dental prophylaxis.

Day 0	Week 4	Week 8	Week 12
Plaque score	Plaque score	Plaque score	Plaque score
Gingivitis score	Gingivitis score	Gingivitis score	Gingivitis score
Stain score	Stain score	Stain score	Stain score
Prophylaxis	New toothbrush	New toothbrush	Scale & clean
			Prophylaxis

9. DRUGS (including the approval status and detailed information, if applicable)

CHX has been approved for use in dental health-care products and as stated above, is used in several commercial mouthwash formulations that are currently available as a non-prescription item in Australia through pharmaceutical retailers.

10. EFFICACY

Extensive animal and human trials over the last 25 years indicate that CHX is safe when used as an oral rinse.

11. ETHICAL CONSIDERATIONS

Part 1

The use of CHX dental care products has no deleterious effects other than minor reversible staining of teeth. The cessation of oral hygiene procedures (such as toothbrushing) for 4 days is not anticipated to have any deleterious effect on the health of the teeth or gums. Any plaque accumulated during that time will be removed at the completion of each part of the study.

Part 2

The second part of the study involves the use of the formulations with normal brushing on established plaque and/or gingivitis. The gingival health can only improve from the baseline with the use of the anti-plaque formulations issued. At the end of the 12 week study, the subjects will receive a professional dental scale and clean, and prophylaxis. Their gingival health will improve as a result.

12. SAFETY & ECOLOGICAL CONSIDERATIONS : NIL

13. OTHER RELEVANT INFORMATION : NIL

14. ANALYSIS AND REPORTING OF RESULTS

Data will be analysed and written in a Masters Thesis, and published in an appropriate refereed journal.

15. REFERENCES

1. Addy, M., Moran, J. et al. (1994). " Chemical plaque control in the prevention of gingivitis and periodontitis." Proceedings of the 1st European Workshop on Periodontitis 244-257. London: Quintessence.
2. Addy, M., Hunter, L. (1987) " The effects of 0.2% chlorhexidine gluconate mouthrinse on plaque, toothstaining and candida in aphthous ulcer patients" . Journal Clincal Periodontology **14**: 267-273.
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16. OTHER ETHICS COMMITTEES TO WHICH PROTOCOL HAS BEEN SUBMITTED : NIL

17. DATE OF PROPOSED COMMENCEMENT : May 1998

18. PROPOSED FUNDING SOURCE : Hamilton Laboratories Pty Ltd

APPENDIX XIII

PROJECT NO: H/.....

Date Received: .....

THE UNIVERSITY OF ADELAIDE
HUMAN RESEARCH ETHICS COMMITTEE

Applications will be considered in terms of the University's guidelines on the ethics of human research, based on the NH&MRC Statement of Human Experimentation (refer application Information Kit which includes list of headings applying to all applications). Submit the application plus 9 duplicate copies to the Secretary, Human Research Ethics Committee, Secretariat, Office of the Vice-Chancellor.

COVER SHEET FOR ATTACHMENT TO APPLICATION FOR ETHICAL APPROVAL OF A RESEARCH PROJECT INVOLVING HUMAN SUBJECTS

APPLICANT: **Surname:** CHONG HIRSCH **Initials:** A.Y.L. R.S. **Title:** DR

DR

DEPARTMENT: DENTISTRY **Tel:**

OTHERS INVOLVED:

(Please indicate if a higher degree candidate)

PROJECT TITLE: THE EFFECTS OF TEA TREE OIL CONTAINING MOUTHWASHES AND TOOTHPASTES ON DENTAL PLAQUE FORMATION AND ON CHRONIC GINGIVITIS.

SOURCE OF FUNDING: Australian Tea Tree Oil Research Institute (ATTORI)

DATE PROJECT TO BEGIN: May 1998

ESTIMATED DURATION OF PROJECT: 12 MONTHS

LOCATION OF RESEARCH: Department of Dentistry, University of Adelaide

BRIEF DESCRIPTION OF THE PLAN/DESIGN OF PROJECT (in lay terms):

There are 2 parts to this study which involves 180 subjects.

Part 1 is a double blind cross over 4 day study involving 30 volunteers, in whom the effects a tea tree oil containing mouthwash will be compared to chlorhexidine and Listerine® and control mouthwashes. The subjects will be monitored for amount of plaque accumulation on the 4th day. The subjects will repeat this procedure for each of the four preparations, with a week between each preparation.

Part 2 is a double blind 6 week study involving 150 subjects (in 5 groups), in whom the effect of tea tree oil containing mouthwashes and toothpaste on pre-existing chronic gingivitis will be compared to Colgate Total® toothpaste.

BRIEF DESCRIPTION OF THE AIMS OF PROJECT (in lay terms):

The commercial production of tea tree oil products began in the 1920's [1]. Several studies have since shown it to have antimicrobial properties [1-8]. The aim of this study is to test oral health care products containing tea tree oil for their effects on the amount of plaque growth and developing gingivitis; and their longer term effects on chronic gingivitis.

ETHICAL IMPLICATIONS OF PROJECT:

Tea tree oil has been incorporated in oral health products for some time. Its antimicrobial effects have been shown in several studies [1-8].

In Part 1 of the study, the cessation of toothbrushing for 4 days will result in plaque formation, which can readily be removed with a professional dental prophylaxis.

Part 2 of the study involves assessing the effects of tea tree oil on existing chronic gingivitis (a condition that is widespread in the population). A further exposure of 6 weeks to chronic gingivitis is not considered to adversely affect these subjects' periodontal status. At the end of the study, the subjects will receive treatment for their gingivitis, which they would probably not have received if they had not participated.

DRUGS:	Will drugs be administered to subjects? If YES - give name of drug(s):	NO
	Will this project be conducted under the Clinical Trials Notification (CTN) Scheme?	NO
	Is Commonwealth Department of Health permission required?	NO
	Has Commonwealth Department of Health permission been obtained?	NO
	Is the administration for therapeutic purposes?	NO
	Dosage: Method of administration:	

SUBJECTS:

Source: University campuses

Age range: 18-40 years old

Selection criteria: Dentate, healthy

Exclusion criteria: Smokers, on medication (prolonged antibiotic therapy, steroids), diabetic, subjects requiring antibiotic cover, patients with pacemakers, hepatic disease, kidney disease, pregnant, lactating females, subjects with periodontitis.

SIGNATURE OF ALL INVESTIGATORS NAMED IN THE PROTOCOL:

Dr A Chong _____

Dr R Hirsch _____

Date:

THE UNIVERSITY OF ADELAIDE HUMAN RESEARCH ETHICS COMMITTEE

PROJECT NO:

APPROVED BY THE COMMITTEE AT THE MEETING HELD ON:

SUBJECT TO:

FOR THE PERIOD UNTIL:

Signed: ..... Date:

Convener

THE UNIVERSITY OF ADELAIDE
HUMAN RESEARCH ETHICS COMMITTEE

1. TITLE

The effects of tea tree oil-containing mouthwashes and toothpastes on dental plaque formation and on chronic gingivitis.

2. INVESTIGATORS & QUALIFICATIONS

Dr A.Y.L.Chong, B.D.S. Postgraduate student in Masters in Periodontics, Department of Dentistry, The University of Adelaide.

Dr R.S. Hirsch, M.D.S., Ph.D., Specialist Periodontist, Senior Lecturer, Department of Dentistry, The University of Adelaide.

3. PURPOSE OF STUDY

Commercial production of tea tree oil (melaleuca alternifolia) began in the 1920's [1]; its antimicrobial properties has be shown in several studies [1-8]. The aim of this study is to test mouthwashes and toothpastes containing tea tree oil for their effects on plaque growth and on existing chronic gingivitis.

4. BACKGROUND

Dental plaque

Dental plaque is a bacterial aggregation on teeth and other oral structures [9]. The first line of defence of the gum against dental plaque includes the rapid and constant shedding of epithelial cell, flushing action of crevicular fluid and its immuniglobulin content, and the passage and activity of polymorphonuclear leukocytes into the gingival crevice.

If plaque is present around the gingival margins of teeth for a sufficient period, gingivitis will gradually develop. Gingivitis is the second line of defence of the gingival tissue to dental plaque bacteria and their products.

Although there is loss of collagen in the tissue adjacent to the sulcus in gingivitis, it is reversed by removing the plaque and re-establishing oral hygiene procedures. The tissues are completely regenerated during the healing phase which occurs within 7-14 days after plaque control is resumed. Gingivitis can be reversed with a professional dental scale and clean., comprising of the mechanical removal of plaque, calculus and stain with scalers; and a dental prophylaxis. A dental prophylaxis involves the polishing of teeth with a rubber cup and a fluoride-containing paste.

Tea Tree Oil

In Australia, the medical properties of tea tree oil were known to the Aboriginals, years before settlement [2]. It is considered to be a non-poisonous non-irritant antiseptic and disinfectant, containing ~55% terpenes and ~7% cineol with an alcohol terpinol [2]. Its ability to penetrate the outer layers of skin due its oily nature may be the reason why tea tree oil is such an effective antiseptic, and possibly as an anti-inflammatory agent.

The extent of the antimicrobial property of tea tree oil is still currently being quantified, the insolubility [8] of the tea tree oil in aqueous being a factor cited

as the reason for difficulties in standard suscetibility tests. The current standard is for 'Oil Melaleuca (terpinen-4-ol type) (AS 2782-1985). It sets a minimum content of terpinen-4-ol of 30% and a maximum 1,8-cineole content of 15% [8]. The main antimicrobial component of tea tree oil is the terpinen-4-ol.

5. PRELIMINARY STUDY (if any); Nil

6. SUBJECTS

These will be 180 healthy, dentate volunteers aged from 18-40 years old. There will be 30 volunteers in the first part (4 days) of this study and the second part of the study (6 weeks) will involve 150 volunteers.

7. EXCLUSION CRITERIA (specific)

Smokers, on medication (prolonged antibiotic therapy, steroids), diabetic, subjects requiring antibiotic cover, people with pacemakers, hepatic disease, kidney disease, pregnant, lactating females, people with periodontitis.

8. PLAN & DESIGN

Part 1: Effect of a tea tree oil containing mouthwash on dental plaque growth

30 healthy university volunteers between 18-40 years of age will participate in this study. This is a double-blind cross over study of 4 preparations. Each subject will go through the same procedure 4 times (using a diffecrent preparation each time).

Subjects will meet the following inclusion criteria of a clear medical history and have at least 20 natural teeth. Medical histories will be taken to exclude the conditions described in item 7.

At the first visit, the subjects will be given the information sheet to read and asked to sign the written consent form. Subjects will then receive a dental examination and a scale and clean, followed by a dental prophylaxis to remove all plaque.

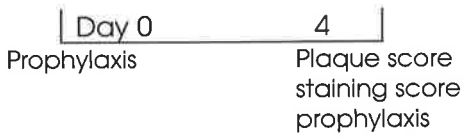
The subjects will be issued with a coded container with one of the following formulations:

	Preparation
1	2% tea tree oil mouthwash
2	Mouthwash base rinse
3	0.12% chlorhexidine mouthwash
4	Listerine mouthwash

The subjects will be required to rinse twice daily with 10ml of each preparation for 1 minute for 4 days. Subjects will be requested not to use any mechanical form of oral hygiene during the 4 days of the study.

On Day 4, the subjects will return to the clinic to have plaque levels scored. Disclosing solution (a vegetable based dye) will be applied to the teeth to make the plaque visible and their teeth will be photographed. Subjects will be

questioned about the acceptability of taste of the products used and any adverse reactions.



Following a 'wash out' period of 1 week, to negate any effects of active ingredients in toothpaste slurries, each subject will return to repeat the procedure with one of the other preparations. This process will be repeated until all the preparations have been tested.

Part 2 : Effects of a tea tree oil containing mouthwash and toothpaste on chronic gingivitis

150 (5 groups, 30 subjects in each group) healthy volunteers between 18-45 years of age will participate in this study. They will meet the following inclusion criteria of a clear medical history, have at least 20 natural teeth and have established chronic gingivitis. Medical histories will be taken to exclude the conditions described in Item 7.

At the first visit (Day 0), the subjects will be given the information sheet to read and asked to sign the written consent form. The subjects will receive a dental examination to assess their plaque levels, extrinsic stain level and gingival health.

Following the baseline examination, the subjects will be categorised according to their age, plaque and gingivitis scores[11,12]. The subjects will then be distributed amongst the following treatment groups so that each group has similar oral health and age characteristics.

The subjects will be issued with one of the following preparations:

Group	*Preparation*
1	Tea tree oil mouthwash
2	Mouthwash base
3	Tea tree oil toothpaste
4	toothpaste base
5	Colgate Total toothpaste

Subjects in Groups 1 & 2 will be asked to brush their teeth as they normally would with a standard toothpaste twice daily; this will be followed by rinsing with 10ml of mouthwash for 1 minute.

Subjects in Groups 3-5 will be asked to brush their teeth with the toothpaste supplied.

On Week 3, the subjects will return to the clinic to have plaque level, extrinsic stain level and gingivitis scored. Disclosing solution (a vegetable based dye) will be applied to the teeth to

make the plaque visible and their teeth will be photographed. Gingivitis will be scored by inspection of the gingiva and recording the BOP. The subjects will be issued with a new toothbrush.

The same parameters will be measured on Week 6, when the subjects will also receive a scale and clean (to return the gingival tissues to health) and a dental prophylaxis.

Day 0 3	Week	Week 6
Plaque score Gingivitis score Stain score Prophylaxis	Plaque score Gingivitis score Stain score New toothbrush	Plaque score Gingivitis score Stain score Scale & Clean Prophylaxis

9. DRUGS (including the approval status and detailed information, if applicable)

Tea tree oil is used in dental health-care products. Several commercial mouthwash formulations are currently available as non-prescription items in Australia through natural and health product retailers.

10. EFFICACY

Tea tree oil containing oral health care products have not been scientifically evaluated.

11. ETHICAL CONSIDERATIONS

Tea tree oil has been incorporated in oral health products for some time. Its antimicrobial effects have been shown in several studies [1-8].

IIn Part 1 of the study, the cessation of toothbrushing for 4 days will result in plaque formation, which can readily be removed with a professional dental prophylaxis.

Part 2 of the study involves assessing the effects of tea tree oil on existing chronic gingivitis (a condition that is widespread in the population). A further exposure of 6 weeks to chronic gingivitis is not considered to adversely affect these subjects' periodontal status. At the end of the study, the subjects will receive treatment for their gingivitis, which they would probably not have received if they had not participated.

12. SAFETY & ECOLOGICAL CONSIDERATIONS : Nil

13. OTHER RELEVANT INFORMATION : Nil

14. ANALYSIS AND REPORTING OF RESULTS

Data will be analysed and written in a Masters Thesis, and published in an appropriate refereed journal.

REFERENCES

1. Humprey, E.I. (1930). " A new Australian germicide" Medical Journal of Australia 417-418.
2. Altman, P. (1988). " Australian Tea Tree Oil" The Australian Journal of Pharmacy **69**: 276-78.
3. Walsh, L. Lonstaff, J. (1987). " The antimicrobial effects of an essential oil on selected oral pathogens." Journal of Periodontology **8**: 11-15.
4. Raman, A., Weir, U. et al. (1995). " Antimicrobial effects of tea tree oil and its major components on Staphylococcus aureus, S. epidermidis and Propionibacterium acnes" Letters in Applied Microbiology **21**: 242-245.
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6. Shapiro, S., Meier, A. et al. (1994). " The antimicrobial activity of essential oils and essential oil components towards oral bacteria." Oral Microbiology Immunology **9**: 202-208.
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9. Dawes, C. (1963). " The nomenclature of the intugments of the enamel surfaces of the teeth". British Dental Journal **16**: 65-68.
10. Page, R.C. (1986). " Gingivitis". Journal Clinical Periodontology **13**: 345-55.
11. Lindhe, J., Rosling, B. et al. (1993) " The effect of a triclosan-containing dentrifice on established plaque and gingivitis" Journal Clinical Periodontology **20**: 327-334.
12. Kanchanakamol, U., Umpriwan, R. (1995) " Reduction of plaque formation and gingivitis by a dentrifice containing Triclosan and Copolymer". Journal Periodontology **66**: 109-112.

16. OTHER ETHICS COMMITTEES TO WHICH PROTOCOL HAS BEEN SUBMITTED : NIL

17. DATE OF PROPOSED COMMENCEMENT : May 1998

18. PROPOSED FUNDING SOURCE : **Australian Tea tree Oil Research Institute**