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The effects of a tea tree oil containing gel on chronic gingivitis

A thesis submitted in partial fulfilment for the Degree of

Master of Dental Surgery (Periodontics)

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Signed Statement

This 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. I give consent to this copy of my thesis, when deposited in the University library, being available for loan or photocopying.

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Steven Soukoulis

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Summary

This double blind, non-crossover study investigated the effect of a tea tree oil containing gel on severe chronic gingivitis. Tea tree oil and its components have a broad spectrum of antimicrobial activity *in vitro* and anti-inflammatory properties *in vitro* and *in vivo*. Its components also have the ability to penetrate epithelial tissues. These properties make it of interest from a periodontal point of view, particularly because there are few published studies about the oral effects of tea tree oil.

A cohort of 49 subjects was recruited into the study after being selected during screening appointments. The selection criteria included healthy, non-smoking males and females with clinical evidence of severe chronic gingivitis and a minimum of 20 teeth (excluding third molars) with moderate to severe periodontitis (at least 4 teeth with pocketing ≥5mm in at least one site around each tooth). The subjects were randomly allocated into three groups. One group was issued with the tea tree oil containing gel, the second with a chlorhexidine containing gel and the third group with a placebo gel which was identical to the tea tree oil gel but did not contain tea tree oil. Subjects were requested to apply the gels to the gingival tissues by brushing twice daily. The following clinical parameters were measured at baseline, 4 and 8 weeks:

- gingival inflammation using the gingival index and papillary bleeding index (directly) and using changes in pocket depth and probing attachment loss (indirectly).
- quantitative measurement of plaque by the plaque scoring system.
- taste rating of each gel.

Data analysis was carried out using a statistical software package (SPSS version 10.05, Chicago). Statistical analyses used were:

- ANOVA to test for variation between the means of each group, producing information regarding statistically significant differences between subject and group effects for each parameter.
- Paired t-tests assessed statistically significant differences within each group for each parameter.
- Scheffé's tests facilitated multiple comparison procedures to isolate the specific differences among several population means.

The p-value used to determine statistical significance was 0.05. If $p = 0.05$, the null hypothesis was rejected.

During the study, funding was lost because the company supporting the research went into receivership. This meant that the original target number for subjects (90) could not be met; the study was continued, but with reduced numbers of subjects. The study took place over approximately 7 months.

The data were separated into different sets by tooth (anterior and posterior teeth), surface (buccal and lingual) and pocket depth (shallow or deep). Few statistically significant changes occurred in most of the clinical parameters regarding differences between group means (ANOVA tests). The papillary bleeding index mean of tea tree oil group in posterior teeth and buccal surfaces was significantly different to the other groups when performing ANOVA tests. T-tests which assessed intra-group variation confirmed that the use of the tea tree oil gel resulted in the greatest change in papillary bleeding index for the all data sets during weeks 0-4 and 0-8. This occurred in 10 time periods out of 15 analysed (3 time periods i.e. weeks 0-4, 0-8 and 4-8 for 5 data sets i.e. posterior/anterior teeth etc). Except for the lingual surfaces, all of these changes were

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- and all the volunteers of the clinical study.

Quote

"Books, like friends, should be few and well chosen".

Joineriana

"Research serves to make building stones out of stumbling blocks".

Arthur D. Little

"Do not confuse motion and progress. A rocking horse keeps moving but does not make any progress".

Alfred A. Montapert

Abbreviations

ANOVA = Analysis of variance

CACDRC = Colgate Australian Clinical Dental Research Centre

CD = Cluster determinant

CEJ = Cemento-enamel junction

CHX = Chlorhexidine

DF/S = Diclofenac/sodium

DMSO = dimethyl sulfoxide

ER = Enhancement ratio

fMLP = N- formyl-methionyl-leucyl-phenylalanine

GI = Gingival index

IL-1β/8/10 = Interleukin – 1 β/8/10

KPF = Ketoprofen

LPS = lipopolysaccharide

LTB4 = Leukotriene B4

MBC = Minimum bactericidal concentration

MIC = Minimum inhibitory concentration

MRSA = Methicillin-resistant *S. aureus*

NO = Nitric oxide

NSAID = Non-steroidal anti-inflammatory drug

$OH^{\cdot}$ = hydroxyl radical

PAL = Probing attachment loss

PBI = Papillary bleeding index

PD = Pocket depth

PG = *P grandifolium*

PGE_2 = Prostaglandin E2

PHP = *P.heptaphyllum*

PHT = *P hebetatum*

PI = Plaque index

PL = *P lewellyni*

PLB = Placebo

PMA = phorbol 12-myristate 13-acetate

PS = *P strumosum*

PSS = Plaque scoring system

TNF α = Tumour necrosis factor alpha

TTO = Tea tree oil

w/v = weight by volume. The mass (in grams) of the substance dissolved in or mixed with 100 millilitres of solution or mixture.

5-FU = 5-fluorouracil

Chapter 1

Introduction

The increasing awareness of the environment and healthy lifestyles has generated demand for so-called herbal or natural products containing extracts of various plants. Tea tree oil (TTO) is one such product that has sparked interest; it is the essential oil derived from the *Melaleuca alternifolia* plant and is commonly found in the north/east of Australia. Australian aborigines have used TTO for centuries to treat a number of ailments/conditions such as abrasions, cuts, colds or flus etc. TTO and its components have broad spectrum antimicrobial activity *in vitro* (Shapiro *et al.* 1994; Carson & Riley 1995a; Cox *et al.* 2000) which includes oral/extra-oral bacterial species, fungi and viruses. The activity against oral bacteria includes cariogenic species and putative periodontopathogens. It has also been shown to possess *in vivo* and *in vitro* anti-inflammatory properties (Juergens *et al.* 1998a; Juergens *et al.* 1998b; Hart *et al.* 2000; Santos & Rao 2000; Brand *et al.* 2001). This gives TTO the potential to affect plaque and gingivitis. An interesting property of the components of TTO is their ability to increase permeation of drugs and other components of essential oils into the skin (Okabe, Obata *et al.* 1990; Williams & Barry 1991; Okabe *et al.* 1992; Cornwell & Barry 1994).

TTO is used in many cosmetic and medicinal products. However, there is very little literature regarding the dental effects of these products; since most are considered to be herbal products, they are not required to undergo the same strict evaluation procedures required by federal bodies such as the Australian Therapeutic Drugs and Goods Administration as do most conventional products.

Products incorporating TTO are numerous, they include bath gels, shampoos, conditioners, moisturisers, sunscreens, deodorants, antiseptics washes and toothpastes. A search of the World Wide Web on "tea tree oil products" usually produces over 45,000 "hits", giving an indication of its current popularity worldwide. However little research evidence exists on the efficacy and safety of these products. For example, no scientific reports of TTO containing toothpastes (sold by various companies) was found in the National Library of Medicine's database (PubMed and Medline).

One TTO containing toothpaste currently on the market (Tea Tree Naturals Toothpaste, Life Plus) has the following ingredients: calcium carbonate, vegetable glycerin, purified water, organic tea tree oil, essential oil of fennel, baking soda, carrageenan (seaweed extract), sodium lauryl sulphate, sea salt. No literature for *in vitro* or *in vivo* testing is available for this product, yet some of its components may present a health hazard to some users. For example, fennel oils and seeds have been associated with seizures and allergic responses (Burkhard *et al.* 1999; Asero 2000) as has TTO and carrageenan for allergic responses (Crellin & Philpott 1990).

Aims of the study

The aims of the study were to determine:

- the effect of a TTO-containing gel used twice a day in unsupervised, untreated subjects with minimal oral hygiene instruction on plaque quantity, gingival inflammation, pocket depth, and probing attachment loss.
- side effects including toxic and allergic responses of the TTO-containing gel.
- the subjective taste acceptability associated with the use of the TTO-containing gel over 8 weeks.

The study design was longitudinal double-blind non-crossover, using three groups; a test group (2.5% TTO-containing gel), a positive control group (0.2 % chlorhexidine gel), and a negative control group (placebo gel identical to the test agent but without TTO). The duration of the study was 8 weeks with clinical assessments performed at 0, 4 and 8 weeks.

The null hypotheses (H_o) for this study were:

- H_o 1: There is no difference between the 0.2% chlorhexidine gel and the 2.5% TTO gel in their anti-plaque effect
- H_o 2: There is no difference between the 0.2% chlorhexidine gel and the 2.5% TTO gel in their anti-gingivitis effect
- H_o 3: There is no difference between the placebo gel and the 2.5% TTO gel in their anti-plaque effect
- H_o 4: There is no difference between the placebo gel and the 2.5% TTO gel in their anti-gingivitis effect

Chapter 2

Literature Review

Tea tree oil

The main active ingredients of TTO are 1,8 cineole and terpinen-4-ol. It contains many more components whose biological activities have not been thoroughly researched. 1,8 cineole is found in other essential oils including the oil of the eucalyptus tree (*Eucalypti aetheroleum*). 1,8 cineole has been shown by several authors to possess anti-inflammatory properties (Juergens *et al.* 1998a; Juergens *et al.* 1998b; Santos & Rao 2000), and is able to penetrate human skin (Williams & Barry 1991). Terpinen-4-ol has similar anti-inflammatory activities as 1,8-cineole (Hart *et al.* 2000; Brand *et al.* 2001) but also has anti-bacterial activity (Carson *et al.* 1995; Carson & Riley 1995a; Hammer *et al.* 1996; Hammer *et al.* 1997; Hammer *et al.* 1998; May *et al.* 2000).

TTO shares a similar range of antimicrobial activity with chlorhexidine, although their mechanisms of action differ (Moran *et al.* 1988; Ostela & Tenovuo 1990; Shapiro *et al.* 1994; Carson & Riley 1995a; Cox *et al.* 1998; Cox *et al.* 2000; Fine *et al.* 2000; May *et al.* 2000). They both have antibacterial, antiviral, and antifungal properties. TTO therefore warrants investigation into its potential to influence dental plaque and gingival inflammation.

The tea tree plant

The tea tree is a small paperbark tree that is a part of the family Myrtaceae; it belongs to two genera, *Leptospermum* and *Melaleuca*. In Australia, the term 'tea tree oil' refers to the oil of the *Melaleuca* species, in particular *M. alternifolia*, *M. linarifolia* and *M. dissitiflora*. *M. alternifolia* is used most often for tea tree oil production in Australia. It

grows on the north coast of NSW, into southern Queensland. Its oil is a clear, colourless to yellow liquid (Barr 1988).

The tea tree plant has narrow leaves found on fine branchlets and fluffy white flowers on the outer canopy in spring. Leaf and fine stem, medium stem, and large stem make up the above ground growth. The tree grows most successfully in damp protected areas, with maximum temperatures ranging from around 30°C in January to 20°C in July and rainfall from 1200-1600mm per annum. Many, but not all tea trees contain essential oils in their terminal branchlets and leaves. Essential oils are volatile materials derived from plants by extraction with solvents, distillation, or expression (compaction). They are most often composed of monoterpene or sesquiterpene hydrocarbons or their oxygenated derivatives, such as alcohols, aldehydes, or ketones. The oils are stored as microdroplets in the glands of plants. After diffusing through the walls of the glands, the droplets spread over the surface of the plant before evaporating into the air. These oils are incorporated into various medicinal and cosmetic products (Crellin & Philpott 1990).

Distillation

Although nearly all of the oil is found in the leaves, a mixture of leaf and stem is used in the distillation process to prevent excessive packing and allow even distribution of steam. Steam distillation is carried out at atmospheric pressure; the steam causes the oil to vaporise from the leaves and stems. The mixture of oil and water vapour passes through a condenser where it is cooled and falls into a separator vessel where the oil rises to the surface to be tapped off (Crellin & Philpott 1990).

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The history of tea tree oil

Every race in the world has practiced the ancient art of herbal medicine. The earliest record of medicinal usage of plants, the Chinese pharmacopoeia, was published in approximately 2700 BC (Fischman 1997). Traditional medicine is still practiced by tribal aborigines in central and northern Australia and this knowledge is now recorded, in most other areas it has been lost irrevocably. Limited reports of aboriginal traditional medicine were published in the mid to late nineteenth century. There were publications in the 20th century by botanists, doctors, pharmacists, teachers, and anthropologists (Barr *et al.* 1988). These were made possible due to increasing contact with the indigenous groups. In the early 1920's, TTO was used by the Australian military in wound dressings since it was found to be more potent than phenolic compounds that were used as antiseptics.

Australian aboriginals do not have a written language and so knowledge of traditional medicine has been passed from generation to generation. The knowledge of aborigines in regards to applications of medicinal plants is astounding. It must be remembered that this knowledge was essential for their survival. The plant-derived medications which include washes, applications, poultices, dressings, lotions or ointments, powders, pastes, spines, drops, steam and smoke, can be used either internally or externally for the alleviation of pain and the promotion of healing (Barr *et al.* 1988). The time of collection of plants is important as they can vary in chemical content according to maturation, the season of the year, or the soil type (Crellin & Philpott 1990). The knowledge of medicinal plants by aboriginal groups varies with location. The same plant is often used in completely different ways in different tribal areas. Different parts

of the plants are considered to be more effective by one tribe than another (Barr *et al.* 1988).

Preparation and use

A common method for preparing bush remedies based on plant matter is boiling. A handful of leaves are crushed in the hands, soaked in water for short a time and then boiled. When cool, a little of the liquid is sipped, and the rest is used as a wash and rubbed on to the chest. The treatment is often repeated as required to give relief from the symptoms, which accompany a cold, flu, fever or congestion of the nose and lungs. When the nose and sinuses are congested, the steam arising from the water boiling is inhaled; sometimes the more simple practice of inhaling the vapor of leaves crushed by rubbing between the hands is followed. It is recommended that young leaves be used as they produce a potent vapour. Where several *Melaleuca* species are found growing in the same area, the species preferred is the one with the most strongly aromatic leaves (Barr *et al.* 1988).

An interesting treatment regime for neonates who show signs and symptoms of colds or flu is the use of leaves from medicinal plants. The leaves (this includes those of the tea tree plant) are placed into a hole in the earth along with part of a termite nest and hot coals. The termite nest and hot coals are pounded until a hot wax is formed, the medicinal leaves are then placed on to the hot wax, which causes them to smoke, rather than burn. The warm leaves are rubbed on to the back of the affected child or the child is placed over the smoke. Sometimes the leaves are applied on to the nursing mother. The oil may enter the bloodstream by penetrating the skin and accumulate in the nursing mother's milk (Barr *et al.* 1988). Components of essential oils have been used in recent studies to treat respiratory disorders (Juergens *et al.* 1998a); 1,8-cineole (a component of

TTO) taken systemically in capsule form was effective in treating respiratory disorders (bronchial asthma).

The traditional use of essential oils applied to the skin indicates their potential to penetrate the epidermis, a unique drug delivery system.

Figure 2.1 Application of prepared leaves (Eucalyptus) to the body of an affected child (Barr *et al.* 1988).

Issues associated with medicinal plants (phytomedicines)

The harvesting, handling and processing of medicinal plants is extremely important in ensuring quality of the end product and minimizing adverse reactions. The time of collection of medicinal plants is an important factor in determining the medicinal activity. At different times of the year, active compounds within plants can vary in concentration. Drying and storage of plants can also effect the concentration of active compounds. Herbs, leaves and flowers are best dried in the shade to avoid colour loss, which has been associated with reduced activity (Crellin & Philpott 1990). With today's large market for medicinal plants, the high demand for plant products

necessitates cultivation outside of optimal conditions, producing variation in concentration of active compounds in the products.

The safety of phytomedicines

When phytomedicines are used with the knowledge of indigenous tribes who have used them for thousands of years, the chances of adverse effects are low. However their use without traditional wisdom or modern science can lead to adverse or even life threatening outcomes. The explosion in sales of plant and herbal therapies has brought many products to the marketplace that do not conform to the standards of safety and efficacy that physicians and patients expect (Pribitkin & Boger 2001). Unfortunately there is no regulatory system that ensures that any of these plant remedies are what they say they are, do what is claimed are safe (Elvin-Lewis 2001). The FDA wrote this about the safety of medicinal plants; "Given the availability of modern medicines with proven effectiveness and safety when used as directed, treating ailments with herbs is both unnecessary and risky" (Elvin-Lewis 2001). While this statement may hold some merit, the FDA does not take into account the side effects accompanying modern medicines.

Other problems include the way in which phytomedicines are used. Many people persist taking medicine for extended periods despite ongoing symptoms rather than seeking medical advice, and take large doses of phytomedicines in the belief that they are absolutely safe because they are natural. The fact that large numbers of plants are poisonous (some in small doses) is unknown or forgotten and that apparently innocuous remedies may have serious long-term health implications (Crellin & Philpott 1990). Phytomedicines can have direct toxic, allergic, narcotic responses and interact with food or medicationss. In the case of TTO, the main concerns are toxicity due to overdose or allergic responses (see following section for details). These can be limited by storing

the TTO sealed in an airtight container away from sunlight which prevents its oxidation. Oxidation of TTO increases the conversion of active components into sensitising or toxic metabolites (Hausen *et al.* 1999).

The chemistry of TTO

TTO is a complex mixture of compounds, consisting predominantly of terpenes which have high biological activity and low irritant qualities (Brophy *et al.* 1989). The oil's activity may depend on the interaction between its numerous compounds since purified terpinen-4-ol was reported to be a less effective antibacterial than the essential oil (Leach *et al.* 1993). Terpenes are widespread in nature, mainly occurring in plants as constituents of their essential oils. Many terpenes are hydrocarbons, but oxygen-containing compounds such as alcohols, aldehydes or ketones (*terpenoids*) are also found. Their building block is the hydrocarbon isoprene, $CH_2=C(CH_3)-CH=CH_2$. Terpene hydrocarbons have molecular formulas $(C_5H_8)_n$, they are classified according to the number of isoprene units (Bailey & Bailey 1991) (Table 2.1).

Table 2.1. Classification of terpenes by the number of isoprene units

Terpene	number of isoprene units
monoterpenes	2
sesquiterpenes	3
diterpenes	4
triterpenes	6
tetraterpenes	8

The major components found in TTO are: terpinen-4-ol, α-pinene, sabinene, γ-terpinene, α–terpinene, limonene, p–cymene, 1,8-cineole, α-terpinolene, α-terpineol, aromadendrene, σ-cadinene, globulol and viridiflorol.

TTO's components and their biological activities

Terpenes are synthesised by the tea tree during normal metabolic reactions. These compounds are vital for the plant's ability to resist certain infections; they may represent the equivalent to mammalian non-specific or innate immune responses. Most microorganisms are unable to colonize living plant tissue. Bacteria and fungi that cause plant disease are usually able to colonize only one species and are restricted to a particular tissue within the susceptible host plant. The ability of plants to minimise or inhibit microbiological colonisation has been attributed to the presence of antimicrobial compounds within challenged tissues (Callow 1983).

Antimicrobial compounds isolated from plants generally fall into two categories:

- *constitutive* compounds which are present in healthy plants. The term constitutive includes compounds which are released from inactive precursors following tissue damage.
- *induced* compounds synthesized from remote precursors following infection.

These compounds have been termed as **phytoalexins** (from the Greek *phyton* = plant and *alexin* = protecting substance). Phytoalexins are "low molecular weight antimicrobial compounds" that are synthesized by, and accumulate in, plants which have been exposed to microorganisms. The primary role of the phytoalexins in these situations may be as a defense mechanism of the living cells, to prevent colonization by secondary invaders of necrotic tissue (Callow 1983).

During primary metabolism eg production of energy *via* metabolism of Acetyl CoA, secondary metabolites are formed leading to phytoalexin biosynthesis (terpenes). The sources of these phytoalexins are important substrates such as phenylalanine, acetyl-

CoA, malonyl-CoA, and mevalonic acid. These compounds are found in numerous plants, pointing to their importance in plant survival.

Antimicrobial action of TTO

Terpenes permit plants to resist infections by invading microorganisms, but are they active against human pathogens? TTO exhibits broad-spectrum antimicrobial activity, which can be principally attributed to terpinen-4-ol (Carson & Riley 1995a). TTO is an effective topical antimicrobial agent when used as a hospital disinfectant (May *et al.* 2000). Kill times were determined using two chemically different TTOs. One was a standard oil and the other was clone 88, which supposedly produces more active agent (terpinen-4-ol) and is less likely to cause skin irritation. The antimicrobial activity of these oils was investigated on Methicillin-resistant *S. aureus* (MRSA), Glycopeptide resistant *enterococci,* Aminoglycoside-resistant *klebsiellae, Pseudomonas aeruginosa, Stenotrophomonas maltophilia.* Both oils had a killing time of less than 60 minutes; the kill rate was not influenced by the Gram stain of the organism.

The antimicrobial activity of TTO *in vitro* was compared to other essential oils such as eucalyptus, manuka, cajuput, manuka, kanuka and niaouli oils (Harkenthal *et al.* 1999). A broth microdilution method was used to assess activity against a number of microorganisms eg *Escherichia coli, Pseudomonas miribalis, Staphylococcus aureus.* TTO had the highest antimicrobial activity overall with a MIC of 0.25%; manuka oil showed the best activity against gram-positive bacteria with MIC values of 0.12%. Harkerthal and coworkers reported that *P. aeruginosa* was not affected by any of the oils, even at the highest concentration tested (4%), contradicting the findings of May *et al.* (2000).

Hammer *et al.* (1996) described the susceptibility of transient and commensal skin flora to TTO. Using a modified broth microdilution method, the MIC of 90% of isolates (MIC90) and the minimum bactericidal concentration of 90% of isolates (MBC90) was tested on a range of skin flora (*Serratia marcescens, P. aeruginosa, S. aureus and capitis* etc). *S. aureus* and most of the Gram-negative bacteria were more susceptible to TTO than the coagulase negative *staphylococci* and *micrococci*, suggesting preferential activity of TTO on transient skin flora while suppressing but maintaining resident flora. Hammer *et al.* (1998) went on to look at the antifungal activity of TTO. TTO showed significant inhibitory and microbicidal activity against 81 *Candida albicans* species and 33 non-albicans *Candida* isolates. TTO's MIC for 90% of all 114 *Candida* isolates was 0.25%. The MBC90 of TTO for *C. albicans* isolates was 0.25%, and 0.5% for 90% of non-albicans *Candida* isolates. A study of the activity of TTO against pathogenic fungi (*dermaphyte* species, *C. albicans* strains and other non-albicans strains and *M. furfur*) found similar MIC to Hammer *et al.* (1998), ranging between 0.5-0.44% Nenoff *et al.* (1996). These values were well below the concentration of TTO found in TTO containing products (5-10%).

Raman *et al.* (1995) explored the difference between the antimicrobial activity of several TTO components (terpinen-4-ol, α-terpineol, α-pinene, 1,8-cineole) against *S.aureus, Staphylococcus epidermis, and Propionibacterium acnes* (bacteria associated with acne). The MIC values of the active components were as follows: α-terpineol < terpinen-4-ol < α-pinene. 1,8-cineole was inactive against these microorganisms. Until recently, terpinen-4-ol was considered the main active antimicrobial agent in TTO, but other components of TTO such as α-terpineol and α-pinene are now recognised to be as important in TTO's antimicrobial activity.

Carson & Riley (1995a) investigated the antimicrobial activity of major components of TTO. Using disk diffusion and broth microdilution methods, 1,8-cineole, 1-terpinen-4-ol, rho-cymene, linalool, α and γ-terpinenes, α-terpineol and terpinolene were tested to determine their activity against a range of bacteria (*S. aureus, C. albicans, E. coli, P. aeruginosa*). Terpinen-4-ol was active against all test organisms, while rho-cymene demonstrated no antimicrobial activity. Linalool and α-terpineol showed activity against all bacteria except for *P. aeruginosa*.

Several studies have investigated the susceptibility of oral bacteria particularly *S. mutans* to essential oils (Namba *et al.* 1982; Charles *et al.* 2000; Fine *et al.* 2000). Few studies have assessed the effects of essential oils on supragingival and subgingival microorganisms (Shapiro *et al.* 1994). Shapiro *et al.* (1994) reported on a survey of the antimicrobial properties of some essential oils and their components (including TTO), singly or in combination, on a large number of laboratory strains of oral bacteria implicated in the development and progression of dental diseases. The bacterial strains examined included:

- Obligate anaerobes: *F. nucleatum, P. gingivalis, P. buccae, P. intermedia, P. nigrescens, S. artemidis, T. denticola, T. vincentii.*
- Facultative anaerobes: *A. viscosus*
- Capnophilic microaerophiles: *A. actinomycetemcomitans, Capnocytophaga* sp., *E. corrodens.*

TTO inhibited the *in vitro* growth of obligate anaerobes and capnophilic microaerophiles at concentrations [0.6 % (w/v). The following MICs/MBCs were determined for TTO: *A. actinomycetemcomitans* 0.11%/>0.60%, *P. gingivalis*

0.11%/μ0.60%, *P. anaerobius* 0.20%/>0.60, *F. nucleatum* > 0.6%/>0.60, *A. viscosus* 0.6%/>0.60, *S. sobrinus* 0.6%/>0.60. TTO had varying inhibitory growth effects when mixed with other essential oils; that is there was synergism or antagonism between oils. When TTO was mixed with peppermint oil, the six bacteria mentioned above were inhibited to varying degrees except for *A. actinomycetemcomitans,* which showed no growth inhibition at all. When mixed with sage oil, thymol and eugenol, all bacteria were inhibited to varying degrees; the TTO and thymol mixture produced the best inhibition (83-100% inhibition) of all bacteria.

Antimicrobial mechanisms of TTO

The cytoplasmic membranes of bacteria and the plasma and mitochondrial membranes of yeast provide a barrier to the passage of small ions such as H, K^+, Na^+ and Ca^{++} and allow cells and organelles to control the entry and exit of nutrients, waste products or water. The permeability barrier role of cell membranes is integral to many cellular functions, including maintenance of the energy status of the cell, other membrane-coupled energy-transducing processes, solute transport, regulation of metabolism and control of turgor pressure (Cox *et al.* 2000). The primary site of the toxic action for cyclic hydrocarbons (aromatics, cycloalkanes and terpenes), is probably the cytoplasmic membrane but the mechanism of the toxicity is still poorly understood (Sikkema *et al.* 1994).

Sikkema *et al.* (1994) studied the effects of cyclic hydrocarbons on liposomes prepared from *E. coli* phospholipids in an attempt to elucidate the changes to bacterial cytoplasmic membrane that occurred on exposure to cyclic hydrocarbons. Cyclic hydrocarbons preferentially resided in the membrane rather than in solution. The accumulation of hydrocarbon molecules caused swelling of the membrane bilayer, and

an increase in membrane fluidity. These effects on the integrity of the membrane caused an increased passive flux of protons and carboxyfluorescein. In cytochrome c oxidase containing proteoliposomes, both components of the proton motive force, the pH gradient and the electrical potential, were dissipated with increasing concentrations of cyclic hydrocarbons. The dissipating effect was primarily the result of an increased permeability of the membrane for protons (ions). At higher concentrations, cytochrome c oxidase was also inactivated. The authors concluded that the impairment of microbial activity by the cyclic hydrocarbons most likely resulted from hydrophobic interaction with the membrane, which affects the functioning of the membrane and membrane - embedded proteins/enzymes (Sikkema *et al.* 1994).

Cox *et al.* (1998) showed that TTO could cause intracellular K^+ leakage and inhibition of glucose dependent respiration in *E. coli*. TTO inhibited respiration in *E. coli, S. aureus* and *C. albicans* cells at minimum inhibitory levels (Cox *et al.* 2000). The possibility that TTO directly inhibited a specific respiratory enzyme or metabolic event cannot be excluded. Minimum inhibitory levels of TTO altered cell membrane structure; this was ascertained by observing the increased uptake of the nucleic acid stain propidium iodide, to which bacterial cell membranes are normally impermeable. Also, leakage of potassium ions commenced immediately upon adding tea tree oil to suspensions of *E. coli* and within 5 min for *S. aureus* cells (Cox *et al.* 2000).

The broad spectrum of activity of TTO and its general membrane-damaging effect probably reflects the rate at which its active components diffuse through the bacterial cell wall and penetrate the phospholipid regions of cell membrane structures. Cox *et al.* (2000) suggested that this mode of action of TTO was similar to that of other broad-

leukocyte activity after stimulation by zymosan and LPS, and measuring nitric oxide (NO) production from stimulated macrophages, with the following results:

- some of the oils inhibited protein extravasation (initial vascular change in inflammation) but did not affect leukocyte proliferation.
- others inhibited LPS induced neutrophil, eosinophil and mononuclear cell accumulation.
- apart from PL which increased LPS stimulated NO production by 49%, all the other oils inhibited NO production by stimulated macrophages ranging from 46-74%. NO produced by macrophages is involved in regulation of vasodilation, and plays an important role in local tissue damage (Kumar *et al.* 1997a).

Monoterpenes can thus affect inflammatory responses at different levels; from the earliest events of changes in vascular permeability to macrophage responses.

Some of the components of TTO are also present in eucalyptus oil and Myrtol standard oil, ie 1,8-cineole, limonene and α-pinene, which decreased the amount of inflammation in respiratory tract infections (Grassmann *et al.* 2000). The radical scavenging and antioxidant properties of these essential oils were investigated by assessing the ability of the oils to react with hydroxy radicals ($OH^{\cdot}$), NO and O_2 radicals and the effect on the degranulation of stimulated neutrophils. The results suggested that Myrtol Standardised and Eucalyptus oil could interfere with inflammatory reactions brought about by over-activated leukocytes. They scavenged the most aggressive radical oxygen species of the $OH^{\cdot}$ radical type and in addition attenuate leukocyte activation (decrease degranulation and therefore the release of hypochlorite ions). These activities may be responsible for preventing oxidative tissue damage *in vivo*.

differentiation was about three times the magnitude of untreated cell expression. Interestingly, both CD 11b, and Fc receptor expression (CD 62/16) expression increased. An increase in CD11b expression represents an increase in differentiation and also increased ability of microbial phagocytosis since CD11b (Mac –1) is a complement receptor. An increase in CD62/16 also represents an also increased ability of microbial phagocytosis since it is a Fc receptor required for antibody binding. These monocytic changes may translate to better microbial clearance by the host due to an increase phagocytic activity by resident monocytes. This may be a favourable activity of TTO since some periodontopathogens are believed to invade human tissues (Schenkein *et al.* 2000; Lux *et al.* 2001).

Toxicology of TTO

With the increase in the use of TTO, reports of sensitivity have followed, numbering over 30 documented cases between 1991-1999 (Hausen *et al.* 1999). Although the majority of the literature comprises case reports rather than controlled studies, several authors have attempted to pin-point the exact mechanism of toxicity and the components of TTO responsible.

At present, the concentration of two components of TTO are regulated by an Australian standard (AS 2782-1985), which stipulates that terpinen-4-ol, the putative antimicrobial component must comprise at least 30% of the oil, while 1,8-cineole, reputedly a skin irritant, must not exceed 15%. While the antimicrobial activity of terpinen-4-ol has been confirmed and is considered to have low toxicity, the role of 1,8-cineole in toxicity is less clear. The lipophilic nature of tea tree oil and its ability to penetrate skin may potentiate its toxicity (Carson & Riley 1995b). No data regarding the oral toxicity of

TTO in humans are available; its acute oral toxicity in rats is 1.9-2.6 ml/kg (Carson & Riley 1995b).

In vitro toxicity of TTO

TTO and its components may adversely affect microorganisms as well as mammalian cells. With increased doses and time exposure, TTO became cytotoxic to promyelocytic cells (Budhiraja *et al.* 1999). In the range of 20–90 μmol/l terpinen-4-ol treated cells were stimulated to produce differentiation markers and Fc receptors (CD 16 and 62). At ranges between 190–380 μmol/l, terpinen-4-ol ceased to have a stimulatory effect and above 380 μmol/l, cell lysis or cytostasis (inactivity) occurred. Soderberg *et al.* (1996) investigated the toxicity of conifer resins and TTO *in vitro*. TTO was only slightly toxic to fibroblasts in concentrations lower than 100 μg/ml but higher concentrations caused a rapid decline in cell viability. TTO was less toxic to epithelial cells than were conifer resins.

In vivo toxicity of TTO

The few *in vivo* reports are confined to accidental ingestion of undiluted TTO. Taken internally, undiluted essential oils are generally irritants to the mucous membranes of the mouth and digestive tract (Seawright 1993). After accidental ingestion of large quantities of TTO, a 17-month-old boy (who ingested less than 10 ml of undiluted TTO) developed ataxia and drowsiness (Jacobs & Hornfeldt 1994). In another report, a 23-month-old boy became confused and unable to walk 30 minutes after ingesting less than 10 ml of T36-C7, a commercial product containing 100% Melaleuca oil (Jacobs & Hornfeldt 1994). The child made a full recovery. Several reports of adverse reactions in adults also exist. A dermatologist reported a case of a 60 year old man who developed a rash after ingesting half a teaspoonful of TTO. The rash affected his feet,

Hausen *et al.* (1999) concluded that if TTO was kept in open or closed containers in direct contact with light, it undergoes photo-oxidation within a few days to several months, leading to the creation of degradation products (ascaridol and 1,2,4 trihydroxy-menthane) that are moderate to strong sensitisers which can initiate the development of allergic contact dermatitis.

Rubel *et al.* (1998) also attempted to ascertain which components of TTO were most likely to cause irritation or allergic responses. Unlike the work carried out by Hausen and coworkers, Rubel used 28 individuals who were not sensitised to TTO. Blocks of paraffin wax impregnated with TTO (containing 1.3 - 28.8% 1,8-cineole), were coated onto adhesive tape and applied on the arm or back of the subjects for 21 days with daily changes of the tape. Three subjects showed a significant response to the patch tests; these subjects were then tested with specific components of TTO for 7 days. Subject 1 elicited a strong response to α-terpinene (+++) and sesquiterpenoid (+++) after eight days. Subjects 2 and 3 elicited strong responses to sesquiterpenoid +++ after 3 and 14 days respectively.

Subject 2 had previously been exposed to TTO whereas the others had become sensitised during the trial. The reaction seen was a true allergic reaction (contact dermatitis) rather than an irritation since the tissues showed dermal oedema and absence of epidermal reaction. There were no reactions to the terpinen-4-ol or the 1,8-cineole in any subjects, even when used in high concentrations. It appears that the sesquiterpenoid fraction of TTO is a potent allergen and the response to α-terpinene was consistent with reactions reported by (Hausen *et al.* 1999).

most effective essential oil at enhancing penetration but was less potent than the corresponding isolated terpene (1,8-cineole). 5-FU was less soluble in the terpenes than in water and the terpenes did not exert their action by increasing partitioning (selective movement) of the drug into the membranes as illustrated by stratum corneum: water-partitioning studies, indicating direct activity on the epithelial membranes.

Okabe *et al.* (1990) investigated cyclic monoterpenes (l-menthol, l-menthone and 1,8-cineole, p-menthane and d-limonene) in the percutaneous absorption promoting effect in rats and skin irritancy in rabbits. Ketoprofen (KPF) was applied to rat skin in gel ointments containing various cyclic monoterpenes. Plasma concentrations of KPF markedly increased with the addition of the hydrocarbons of cyclic monoterpenes such as trans-p-menthane and d-limonene, whereas no significant enhancing effect was observed in the cases of other terpenes such as l-menthol, l-menthone and 1,8-cineole, contradicting the results seen by (Williams & Barry 1991). Irritancy of the cyclic monoterpenes was tested using ethanol containing 2% of the monoterpenes was applied to the dorsal skin. No adverse changes were noted.

Okabe *et al.* (1992) went on to investigate the percutaneous absorption of ketoprofen (KPF) from gel patches containing d-limonene and ethanol was investigated in rats. Plasma levels of KPF varied with the kind of polymers which constituted the gel patch, also the amount of KPF permeating through the rat skin from the gel patch was well correlated with that of ethanol. Permeations were enhanced with increase in the amount of d-limonene distributed from the vehicle to the skin tissue. Obata *et al.* (1990) investigated the promoting effect of cyclic monoterpenes on the percutaneous absorption of diclofenac sodium (DFS) in gel form, in rats. Of five cyclic monoterpenes examined, l-menthol was most effective. Plasma concentrations of diclofenac (DF)

increased with increasing amounts of l-menthol in the gel ointment refuting the results of Okabe *et al.* (1990). The difference between these studies' results may have been due to varying study methodology.

Recently, El-Kattan *et al.* (2000) investigated ketoprofen enhanced permeation of mouse skin i*n vitro* by essential oil components (limonene, nerolidol, fenchone, and thymol). The authors found that limonene caused the highest increase in ketoprofen permeation. No correlation was established between terpene lipophilicity and ketoprofen skin content values at 24 hr. Ethanol had a synergistic effect on the enhancing activity of the terpenes. Increasing the concentration of ethanol from 10% to 50% was associated with increased permeation of ketoprofen.

Possible mechanism of penetration enhancement

The lipid-protein-partitioning theory has been proposed to explain the modes of action of penetration enhancers based on molecular and solvent changes in the stratum corneum. According to this theory, accelerants may act by one or more of three main mechanisms:

- disruption of the highly ordered lipid structure between the corneocytes, so increasing intercellular diffusivity,
- interaction with intracellular protein to promote permeation through the corneocyte,
- and increased partition of the drug or a coenhancer into the tissue (Williams & Barry 1991).

Terpenes were thought to have interacted with and modified intercellular stratum corneum lipids, disrupting their highly ordered structure to increase diffusivity. Rather than altering a drug's nature in order to facilitate diffusion, terpenes affect cell

membranes in such a way as to increase the diffusion of a drug through the epidermis (Williams & Barry 1991).

An important property of a penetration enhancer would be reversibility of its effects. The reversibility of action of the terpenes has been verified. Under diffusional conditions, samples of human epidermal membranes were treated with carveol, menthone, or 1,8-cineole for 12 hours, while control samples were treated with 0.002% aqueous sodium azide solution. After prolonged washing, the terpene-treated membranes showed no significant increase in the diffusion of 5-FU compared to untreated epidermis. This invariance suggests that the terpenes did not extract significant amounts of barrrier lipid from the tissues.

Effect of essential oils on oral health

There are only two published studies assessing TTO's effects on oral bacteria and on plaque formation *in vivo*. Rogers & Gully (1999) showed that a TTO containing mouthwash was effective in decreasing salivary bacterial counts for approximately ½ hour. This decrease was comparable to the CHX control, but after 6 hours, salivary bacterial levels nearly returned to normal whereas the activity of CHX persisted. This may indicate the lack of substantivity of TTO components. Similar results were seen in a clinical trial of TTO mouthwash. (Arweiler *et al.* 2000) used a crossover study design with a washout period of 3 days in 8 subjects. In the first week, water was used as a mouthwash in the absence of oral hygiene for 4 days, CHX mouthwash was used in the second week and TTO mouthwash in the last week. Plaque index and plaque areas were scored, as was vital fluorescence to detect vital bacterial colonies. The TTO containing mouthwash had no effect on the quantity or quality of supragingival plaque.

The only oral hygiene product available for comparison is Listerine®, since TTO and Listerine® contain some common essential oils. It is however used as a mouthwash rather than gel and so their variation in composition and physicochemical properties will make their activity different. Nonetheless, Listerine® contains essential oils ie thymol, menthol and cineole (eucalyptol) which are also present in TTO. A review of its activity and mechanisms of action may give insight into those of TTO.

Oral health products containing Listerine® include pre-brushing rinses, toothpastes and a large variety of mouthwashes. The active ingredients of Listerine® products are: thymol 0.064%, eucalyptol (1,8 cineole) 0.092%, methyl salicylate 0.060%, menthol 0.042% (percentages for mouthwash). Other ingredients include: water, alcohol (21.6%), sorbitol solution, flavor, poloxamer 407, benzoic acid, sodium saccharin, sodium benzoate and FD&C green # 3. The oral effects of Listerine® have been comprehensively examined in the literature. Its activity ranges from antibacterial, antiviral, antifungal to some reports of anti-inflammatory properties.

Antimicrobial activity

The effectivcness of Listerine® in controlling supragingival plaque and gingivitis has been demonstrated in numerous short and long term clinical trials (Axelsson & Lindhe 1987; Mankodi *et al.* 1987; DePaola *et al.* 1989; Overholser *et al.* 1990; DePaola *et al.* 1996). Its activity is inferior to CHX, probably due to its lack of substantivity (Gultz *et al.* 1998). The primary mechanism underlying the clinical activity of Listerine® mouth rinse is thought to be its microbiocidal activity. Numerous studies have demonstrated

this property of Listerine® *in vitro* and *in vivo* (Ciuffreda *et al.* 1994; Fine *et al.* 1996a; Kaim *et al.* 1998; Fine *et al.* 2000; Pan *et al.* 2000). Some of the oral microorganisms studied were: *A. viscosus, P. intermedia, C. albicans, L. casei, F. nucleatum, P. aeruginosa, S. sanguis, S. mutans, A. actinomycetemcomitans, E. corrodens,* and *C. rectus* (Pan *et al.* 2000).

In vitro, Listerine® can kill a wide range of microorganisms within 30 seconds of exposure in the presence of serum (Ross *et al.* 1989). A single rinse of Listerine® reduced levels of recoverable bacteria in saliva compared to a negative control rinse for periods of up to 5 hours (DePaola *et al.* 1996). Mouthrinses containing Listerine® can produce significant reductions in Gram-negative bacteria in the gingival crevicular region and on the dorsum of the tongue (Pianotti & Pitts 1978; Pitts *et al.* 1981; Pitts *et al.* 1983). These studies are relevant because the tongue and saliva are considered sources of the bacteria that colonise teeth and gingival tissues, thereby contributing to plaque formation (Liljemark *et al.* 1997).

Biofilms and Listerine®

In considering the mechanism of action of antiplaque/antigingivitis products, their action against biofilms could be important since dental plaque is considered to be a biofilm (Darveau *et al.* 1997). Because of the protection biofilms may offer microorganisms, the *in vitro* microbicidal activity of antimicrobials may not be indicative of their activity in the mouth. To address the question of if and how Listerine® acts when confronted with the challenge of penetrating a bioflim and killing the component bacteria, Pan *et al.* (2000) conducted an *in vivo* study using a vital cell staining method. In the past, vital staining methods have produced inconsistent results when compared to clinical plaque reduction findings in the same studies (Brecx *et al.*

1990; Brecx *et al.* 1992). The staining method uses two different dyes, one that stains cells irrespective of vitality (SYTO 9), the other (propodium iodide) stained cells with damaged membranes (indicating damaged or dead cells). With the appropriate staining mixture, bacteria with intact membranes stained fluorescent green and damaged or dead bacteria stained fluorescent red. Listerine® mouthrinse was able to penetrate the plaque biofilm and exert a bactericidal effect on the organisms contained within (Pan *et al.* 2000).

How does Listerine® exert is antimicrobial effects?

The primary mechanism of action of Listerine® is thought to involve effects on the bacterial cell wall. Investigations by Kubert *et al.* (1993) support this view. The group investigated the effect of exposure to Listerine® on the surface morphology of a representative panel of oral microorganisms using SEM. *A. actinomycetemcomitans (Aa), F. nucleatum, S. sanguis, A. viscosus and C. albicans* were immersed in Listerine® antiseptic for 30 seconds, washed in sodium phosphate buffer, fixed, dehydrated and prepared for SEM study. All microorganisms demonstrated changes in cell surface morphology, characteristic of ultrastructural changes seen in reversible cell injury (Kumar *et al.* 1997b) when compared to untreated bacteria. The surface changes reported might explain some of Listerine®'s effects at sublethal concentrations (ie decreases in growth rates and coaggregation of bacteria), since these functions are associated with surface receptors (Kubert *et al.* 1993).

The effect of sublethal exposure of oral bacteria to Listerine® has received attention from Marsh (1992) and Fine *et al.* (1996a). After rinsing, the active agent in a mouthwash may be briefly at levels above its MIC, but thereafter, it will be desorbed from oral surfaces and operate at sub-lethal concentrations. At these levels, agents can

be effective by inhibiting metabolism (eg, acid production, protease activity), and slowing bacterial growth (Marsh 1992). Sublethal levels of Listerine® slow the growth rate of pioneer bacteria (Fürgang *et al.* 1992) as well as interfering with intrageneric coaggregation of a variety of oral bacteria (Kurik *et al.* 1992). Fine *et al.* (1996a) investigated the effects of sublethal levels of Listerine® on selected activities of representative plaque microorganisms using *in vitro* models. Significant effects included reduced intergeneric coaggregation, increasing bacterial generation time, and extracting endotoxin from Gram-negative bacteria. These processes are very important to the bacterial cell's abilities to form biofilms and induce an inflammatory response in the host.

Anti-inflammatory activity of Listerine®?

Many chemotherapeutic agents such as Listerine®, sanguinarine or chlorhexidine have been used to decrease dental plaque formation, thereby indirectly reducing gingival inflammation. However gingival inflammation may be decreased independently of plaque reduction. For example, while chlorhexidine was more effective in inhibiting dental plaque accumulation than sanguinarine, Meridol® or Listerine® (Brecx *et al.* 1990; Quirynen *et al.* 1990), Listerine® was more potent in reducing gingival inflammation (Brecx *et al.* 1990). A possible explanation for Listerine®'s ability to decrease inflammation is that it has antioxidant properties, and inherent anti-inflammatory activity. Firatli *et al.* (1994) subjected bovine brain tissue, which is an extremely potent oxidative medium very rich in unsaturated fatty acid containing lipids, to Listerine®, sanguinarine, cetyl pyrimidine, chlorhexidine, tetracycline and doxycycline. Listerine® and sanguinarine displayed the most effective anti-inflammatory activity next to doxycycline. At smaller volumes (0.1–0.2 ml),

doxycycline had the best anti-inflammatory properties but once the aliquots of agents were increased (> 0.2 ml), both Listerine® and sanguinarine equalled the activity of doxycycline. The authors were not sure how the agents produced these results but suggested that they may have interacted with biological membranes and with the oxidative process. The anti-inflammatory activity of Listerine® may be related to some of its components which possess anti-inflammatory properties (see pages 17-21) and the ability to diffuse through human epithelium (see page 26).

Virulence factors of several oral bacteria may contribute to the periodontal inflammatory process. *S. sanguis* and *P. gingivalis* have been shown to induce platelet aggregation *in vitro*. Once platelets aggregate, they become activated and can produce inflammatory mediators (histamine, prostaglandins, serotonin) (Kumar *et al.* 1997a). One study looked at the effect on the platelet-aggregating activity of these organisms when treated with Listerine® (Whitaker *et al.* 2000). In all cases, the control-treated organisms induced platelet aggregation, treatment with Listerine® completely inhibited the platelet aggregating activity of *P. gingivalis* and of *S. sanguis*. The authors suggested that the effect of the essential oil mouthrinse was a function of the oils' ability to interfere with bacterial cell surface-associated activities.

Chlorhexidine

Chlorhexidine is a bisbiguanide antiseptic; it is a symmetrical molecule consisting of 4 chlorophenyl rings and 2 biguanide groups connected by a central hexamethylene bridge. The compound is a strong base and is dicationic at pH levels above 3.5, with 2 positive charges on either side of the hexamethylene bridge. The dicationic nature of

chlorhexidine makes it extremely interactive with anions. This is the key to its efficacy, safety, local side effects and difficulties with formulation in products (Addy 1997).

Chlorhexidine was developed in the 1940s by Imperial Chemical Industries, England and marketed in 1954 as an antiseptic for skin wounds. Later, the antiseptic was more widely used in medicine and surgery including obstetrics, gynaecology, urology and pre-surgical skin preparation for both patient and surgeon (Addy 1997).

Its use in dentistry was initially confined to pre-surgical disinfection of the mouth and in endodontics. Today, despite the excellent plaque inhibitory properties of chlorhexidine, widespread and prolonged use of the agent is limited by its local side effects (poor taste, altered taste sensation, staining with prolonged use, increased calculus deposition). Moreover, the antiseptic is of limited value in the therapy of established periodontitis because of its lack of ability to penetrate into periodontal pockets when used as a mouth wash.

Antimicrobial activity

Chlorhexidine is effective against a wide range of microorganisms including: Candida (Giuliana *et al.* 1997), aerobes and anaerobes (Schiott *et al.* 1970; Emilson 1977), Gram positive/negative bacteria (Schiott *et al.* 1970; Emilson 1977), and viruses such as Herpes Simplex, Influenza and Hepatitis B viruses (Harbison & Hammer 1989; Bernstein *et al.* 1990; Russell & Furr 1991).

Plaque inhibition by chlorhexidine was first investigated in the early 1960's, and definitive clinical studies were performed by (Schiott & Löe 1970; Schiott *et al.* 1970). These studies showed that rinsing for 60 s, twice per day with 10 ml of a 0.2% (20 mg dose) chlorhexidine gluconate solution, or topical application of 0.2% chlorhexidine

gluconate inhibited plaque regrowth, the development of gingivitis and a decrease in salivary bacteria in the absence of normal tooth cleaning (Addy 1997). Since then many studies have investigated the activity and effects of chlorhexidine.

Mechanism of action

CHX is a potent antibacterial agent; it binds strongly to the negatively charged bacterial cell membranes. At high concentrations CHX is bactericidal, acting as a detergent that attacks the bacterial cell membrane leading to hypertonic disruption of the cell (Fine 1988). At low concentration, CHX interferes with potassium ion gradients along the cell surface (Hugo & Longworth 1965), and/or ATP synthase activity (Fine 1988). In the mouth, CHX readily adsorbs to surfaces, including pellicle-coated teeth. Once adsorbed, CHX is slowly released, resulting in a persistent bacteriostatic action, lasting in excess of 12 hours (Schiott & Löe 1970). The plaque inhibitory action is derived from the CHX adsorbed to the tooth surface; the molecule attached to pellicle by one cation leaves the other cation free to interact with bacteria attempting to colonize the tooth surface (Addy 1997). CHX also inhibits the production of matrix metalloproteinases (MMPs) *in vitro* (Gendron *et al.* 1999), although how clinically significant this property is may be questionable since chlorhexidine does not penetrate the gingival tissues or pocket when used as a mouthrinse and does not remain in the pocket environment for long when delivered subgingivally (Loesche 1999).

Studies of CHX gel

CHX gel can be delivered on a toothbrush or in trays. Its distribution in the mouth when applied on a toothbrush as a 1% chlorhexidine gel was poor in 24 subjects who brushed the buccal tooth surfaces twice daily for two 4-day periods (Saxen *et al.* 1976). The gel had no more effect on plaque growth lingually than the control toothpaste. The lack of

effect of the gel was thought to be due to rapid adsorption of CHX on to the first surface of contact and the lack of activity in saliva due to its binding with salivary proteins and desquamating epithelial cells, leaving little active agent available elsewhere (Bonesvoll *et al.* 1974).

Bonesvoll (1978) found that the retention of 1 g of 1.0 % CHX gel was equal to that of 0.1 % CHX mouth rinse. Retention was determined by rinsing with deionised water after using CHX and measuring the amount present in the expectorant. After subjects rinsed with water, the amount of CHX was reduced but more than 70% of the initially retained CHX was left after three 10-second rinses, indicating that CHX was firmly adsorbed. The binding of CHX gel in the mouth during brushing with CHX gel occurred rapidly, as indicated by a high retention rate after only 15 seconds of brushing. Brushing time had very little effect on its retention in the time range tested, although the dose of the CHX did (the higher the dose, the greater the retention). The frequency of brushing and the amount of gel used may be important determinants of the clinical effect since they determine the dose of the drug administered.

A decrease in plaque formation is a common finding in studies of CHX gel but the response regarding decreased gingivitis has not been as consistent. For example, a gel containing 0.5% CHX gluconate was tested for its ability to affect plaque formation and the gingival status of 12 male dental students in a controlled double-blind cross-over study. The CHX gel significantly reduced plaque formation when compared with a placebo gel, but the improvement in gingival health was not statistically significant (Lennon & Davies 1975). Bain & Strahan (1978) reported similar findings in 30 patients who took part in a 4 week double-blind clinical trial. Following initial oral hygiene instruction and scaling, half the patients were instructed to brush with a 1%

CHX gel in the evenings, the other half used a placebo. A standard dentifrice was used by all subjects in the morning. There was a marked improvement in all parameters recorded in both groups, and although final plaque scores showed a statistically significant difference in favor of the test group, there was virtually no difference in rate or degree of resolution of gingivitis. The use of standard dentifrice was only partially successful in preventing staining.

While there are several CHX gel studies assessing plaque and gingivitis prevention or resolution, few have assessed the effect of supragingival application in the treatment of periodontitis, possibly due to its poor penetration of deep pockets. When applied subgingivally (usually with a syringe), the results have varied. After application of 1% CHX gel once daily for two consecutive days to subjects with periodontitis (untreated), there was no significant change to the composition of the microflora, plaque, gingival indices or pocket depth compared to the placebo (Kalaitzakis *et al.* 1993). This study was limited because of its short duration and small sample size.

Comparison of essential oils and chlorhexidine's effect on plaque and gingivitis

McKenzie *et al.* (1992) compared the efficacy of a 0.12% chlorhexidine mouthrinse and Listerine® on plaque accumulation and gingivitis in mentally handicapped adults over a one- year period. Twenty-seven institutionalised mentally handicapped adults participated. Gingival index (GI), plaque index (PI), and probing depths (PD) were recorded and an ultrasonic scaling was performed (Baseline 1). GI, PI, and PD were again recorded after 2 weeks (Baseline 2). The patients rinsed twice daily under supervision with their assigned mouthrinse while maintaining their attempts at daily brushing. GI and PI were recorded at monthly intervals for 12 months, while the PD was recorded only at Baseline, 1 and 2 and 12 months. A statistically significant decrease in

the probing depth occurred as a result of ultrasonic scaling; however, no significant changes in the PI or GI occurred. Both mouthrinses produced a significant improvement in the GI after one month but was indicative of disease. Over the 12 months, no further improvement in GI occurred. A statistically significant improvement in the PI occurred in the chlorhexidine group at one month, but returned to Baseline 2 levels over the 12 months. No improvement in the PI occurred consequent to the use of Listerine®. The probing depths remained the same over the 12 months.

The physicochemical properties of antiseptic oral gels

There are very few studies of the physicochemical properties of antiseptic oral gels, but one study does address this topic in reference to mouthrinses. Perdok *et al.* (1990) evaluated the physicochemical properties (surface tension, *in vivo* enamel contact angle, viscosity, penetration coefficient, acidity and buffer capacity) of eight commercially available mouthrinses (including chlorhexidine and Listerine®). The penetration coefficient, determined by the surface tension, contact angle and viscosity, is a measure of the ability of a liquid to penetrate into a capillary space, such as interproximal regions, gingival pockets and pores. Listerine® had the highest viscosity. CHX had the highest penetration coefficient, and Listerine® one of the lowest. The author suggested that for a mouthrinse to enter a pocket, its penetration coefficient had to be very high (close to that of water), but no mouthrinses achieved the coefficient required to do so (Perdok *et al.* 1990).

The oral clearance of 4 CHX solutions and 2 chlorhexidine gels was investigated by (Borer *et al.* 1978). Two 0.2% CHX solutions, Plak-Out liquid, a 10% alcoholic CHX solution and an aqueous ICI-Hibitane solution were compared with two CHX gels, Corsodyl gel (0.1%) and a test CHX gel (1.0%). Thirty subjects participated in

supervised rinsing with CHX solutions and toothbrushing with gels carried out at weekly intervals to eliminate carry-over effects. Mixed saliva samples were taken 7.5, 15, 30, 60 and 120 minutes following each administration and were analysed spectrophotometrically. The retention rates of the mouthwashes was superior to that of the gels; the 0.1% Corsodyl gel had a better retention rate than the more concentrated laboratory gel.

In summary, TTO is a broad-spectrum antimicrobial agent which is effective against oral/extra-oral bacterial species, fungi and viruses. The activity against oral bacteria includes cariogenic species and putative periodontopathogens. The in vitro evidence would indicate that TTO may be an effective anti-plaque agent, however the only clinical study using TTO in a mouthwash (and other essential oil containing products such as Listerine® mouthwashes shows it to have limited activity against plaque. The antimicrobial mechanism of TTO appears to be related to its ability to perturb cytoplasmic membranes. The main active components related to this activity are terpinen-4-ol, α-terpineol and α-pinene.

TTO and its water soluble components have also exhibited anti-inflammatory properties in vitro. They can induce an increase in phagocytic ability, decrease inflammatory cytokine release of stimulated monocytes and decrease release of ROS and scavenge them when released from activated neutrophils.

However, TTO can produce adverse effects when the product being used is not stored correctly or applied/ ingested in large amounts/concentrations. TTO applied to the skin or ingested can cause severe toxic and allergic reactions. Several of the oxidation products of its components have been recently identified as the primary agents for

adverse reactions. These toxic/allergenic components increased when the TTO was exposed to light over long periods.

An interesting ability of terpenes which are found in TTO and other essential oils is the ability increase the penetration of drugs and other components in the essential oils through epithelium. This property may allow for administration of TTO to the gingival tissues in a novel way.

Chapter 3

Materials and methods

Adelaide University's Human Research Ethics Committee gave ethical approval for this study. The approval number was H/47/99.

Sample selection

Males and females aged between 18-60 years, living in Adelaide the state capital of South Australia, were recruited from the general population according to set criteria (Table 3.1). Subjects were recruited by advertisements in The Advertiser and The Messenger newspapers (Appendices I & II). Flyers were also distributed around the clinics of the Adelaide Dental Hospital and the Adelaide University campus targeting dentists and potential subjects (Appendices III and IV).

Clinical screening and subject recruitment

Subjects interested in participating were asked to contact the Colgate Australian Clinical Dental Research Centre (CACDRC) located in the Adelaide Dental Hospital. They were screened over the phone by reception staff who outlined the selection criteria to the potential subject and give a brief explanation of the study. If the selection criteria were met, the subject was given an appointment for clinical screening.

At the clinical screening session, the reception staff handed potential subjects a questionnaire (Appendix V) and an information sheet outlining the aims and protocols of the study (Appendix VI). The researcher interviewed each subject, confirming that the information given was correct i.e. name spelling, address and medical and treatment history (periodontal), and the protocol and agents to be used.

Table 3.1. Inclusion and Exclusion criteria.

Inclusion criteria	Exclusion criteria
▪ Males or females between the ages of 18-65 years individuals with: ▪ Moderate to severe periodontitis ▪ (At least 4 teeth with pocketing ≥5mm in at least one site around each tooth) ▪ At least 20 teeth (excluding third molars) ▪ All participants had to read an information form, receive a verbal explanation of the study and sign an ethical consent form.	▪ Current smokers ▪ Drug exclusion: ▪ Steroids, NSAIDs, Dilantin ▪ Prolonged systemic antibiotic therapy or antibiotics used for at least 7 days in the last 6 months ▪ Subjects requiring antibiotic cover for: ▪ Artificial valves, congenital heart defects i.e. pathological murmurs ▪ People with pacemakers **Systemic diseases:** ▪ Diabetes, hepatic disease, kidney disease, rheumatoid arthritis, pregnant or lactating females. ▪ Periodontal therapy in the last 6 months ▪ Known allergies to tea tree oil.

A clinical examination was carried out to ascertain the number of teeth present, the presence of at least 4 teeth exhibiting pocketing ≥ 5mm in at least one periodontal site out of four (mesio-buccal, buccal, disto-buccal and palatal). The gingival tissues around these teeth also had to show significant signs of bleeding on probing and inflammation. If an individual met the selection criteria, the assistant recorded the information on a data collection form. If the subject agreed to participate in the trial, a consent form (Appendix VII) was signed by the subject, the investigator and an independent witness. An appointment note was given to the subject with dates of their clinical assessments (weeks 0, 4, 8). Individuals who did not meet the selection criteria received a Colgate

Oral Care kit (containing a toothbrush, toothpaste, dental floss and a pamphlet on oral care) and were thanked for expressing their interest.

The study protocol

The study was a longitudinal, non-crossover double blind clinical trial set over 8 weeks with three clinical assessments within the 8-week period. The study was carried out as follows:

Week 0 (baseline)	Week 4	Week 8
Photographs	Photographs	Photographs
Pocket depths	Pocket depths	Pocket depths
Probing attachment loss	Probing attachment loss	Probing attachment loss
Gingival index	Gingival index	Gingival index
Papillary bleeding index	Papillary bleeding index	Papillary bleeding index
Stain & Plaque score	Stain & Plaque score	Stain & Plaque score
Photographs	Photographs	Photographs
New toothbrush issued	Taste rating	Taste rating
	New toothbrush issued	Scale & Clean
	New tube of gel issued	Cash gratuity

Week 0

On the first visit (Week 0), a pamphlet of instructions (Appendix VIII) was given to each subject.

- Subjects were asked to brush their teeth with their issued product twice daily for at least 2 minutes. Subjects were given a pamphlet describing toothbrushing methods. It was stressed that contact of the gel with all tooth surfaces and the adjacent gingival tissues was important.
- Subjects were asked to refrain from using proprietary toothpastes or mouthwashes during the study.

- If subjects fell ill and/or had taken antibiotics or became pregnant, they were requested to inform the CACDRC as they would no longer able to be included in the trial.

A toothbrush (Colgate Australia, soft bristles) and tube of gel (chlorhexidine, placebo, or TTO) sealed in a paper bag labelled with a code number was given to the subject. Random distribution of the gels was carried out by separating the tubes into three boxes of 30 and labelling the boxes I (numbers 1-30), II (numbers 31-60), and III (numbers 61-90). The first subject in the study received a gel from box I, the next subject received a gel from box II and the third from box III and so on.

The researcher had no knowledge of the content of any tube which were supplied and numbered by the company funding the research. The codes were not divulged to the researcher until after the data had been analysed.

The subjects' teeth were photographed using a Canon EOS 50 camera fitted with a 100mm macro lens and a ring flash. The film used was Fujichrome Sensia II (100 ASA). Photographs of each subject's gingival tissues were taken in centric occlusion and others specifically targeted areas of inflamed gingivae under investigation.

Clinical examination

All oral examinations were conducted by the researcher. FDI notation was used during the examinations. Dental assistants entered data on a custom written computer entry program, which was linked to a data collection sheet. It consisted of sheets with the appropriate indices for the clinical examination (Appendix IX). The following clinical measurements were made (in the order that they appear) to assess subjects' periodontal status at weeks 0, 4 and 8.

Pocket depth (PD)

Sterile, pressure sensitive probes (Pro-Dentec, Batesville, Arkansas, USA) with a diameter of 0.55mm which required uniform pressure of 20g to displace the mobile component of the probe were used to measure pocket depth (crest of the gingival margin to the base of the periodontal pocket). To minimise intra-examiner variability when probing the interproximal area, the interproximal contact point/embrasure was used as a guide in terms of horizontal and axial alignment of the probe. On the mid-facial or mid-oral aspects, the long axis of the tooth was used to orientate the probe.

Probing attachment loss (PAL)

PAL (distance in mm from the CEJ to the base of the periodontal pocket or gingival sulcus) was measured at the same time as pocket depth. Where the CEJ was not visible, PAL was difficult to determine. To minimise intra-examiner error at these sites, the CEJ of neighbouring sites was used to estimate the position of the CEJ on the tooth being measured. If this was not possible, the gingival margin of the tooth and neighbouring teeth provided a reference point for the estimation of the CEJ's position. Changes in PAL, rather than its extent, were important in the current study. PAL and PD were therefore scored as the same when the CEJ was not visible. Hyperplastic tissue was determined by comparing neighbouring gingival contours and scoring PD greater than PAL by estimation of the extent of hyperplasia.

Gingival Index (GI)

The GI (Löe 1967) was used to assess gingival health/inflammation. This was determined after PD and PAL measurements were taken.

Table 3.2. Gingival Index

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

Papillary bleeding index (PBI) Mühlemann (1977) (summary from Fischman 1988)

Gingival health /inflammation was also assessed by using the PBI (Table 3.3). Probing of the interproximal gingival tissue was performed on the facial and oral surfaces. Measurement of extent of bleeding was determined and scored.

Table 3.3 Papillary Bleeding Index (PBI)

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

Plaque Scoring System (PSS) (Quigley & Hein 1962)

Plaque accumulation was measured by disclosing plaque with 12 drops of disclosing solution containing 1% w/v erythrosine (Disclogel, Colgate Australia) and requesting that subjects swirl the solution in the mouth for 60 seconds and rinse the mouth with water to allow for maximum contrast between the stained plaque deposits and clean

tooth surfaces. The plaque scoring system is a modification of the Quigley & Hein (1962) suggested by (Fischman 1988)

Table 3.4. Plaque Scoring system

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

Week 4

The same parameters were measured as at Day 0 and photographs were taken. Each subject was asked to rate the gel's taste (Table 3.5).

Table 3.5 Taste rating

1	Poor taste
2	Average taste
3	Good taste
4	Very good taste

Subjects were also issued with a new tube of gel with the same code and a new toothbrush.

Week 8

The same parameters were measured as for week 4. The subjects also received a scale and clean, a dental prophylaxis and cash gratuity ($50). Subjects signed a form to confirm that they had received $50, a scale and clean and a dental prophylaxis.

Intra-examiner standardization

Prior to starting the trial, the examiner carried out repeated examinations on 6 individuals, one day apart to establish that the clinical measurements were made with uniform interpretation and consistency.

Statistical methodology

No prior sample size or power calculation planning was done. Many studies assessing CHX gel have used low numbers of subjects usually ranging from 10-20 subjects, but more recent studies have increased the number to over 40 (Butler & Heslin 1975; Lennon & Davies 1975; Hoyos *et al.* 1977; Bain & Strahan 1978; Joyston-Bechal *et al.* 1984; Almqvist & Luthman 1988). It was decided that an estimated sample size of 30 subjects per group would be sufficient to investigate the effect of topical application of TTO on moderate to severe periodontitis. The study commenced in March 2000 and data collection was completed by late October 2000. Due to loss of funding half way through the project, the number of subjects had to be reduced. The statistical analysis of the data was carried out using a computer software program (SPSS version 10.05, Chicago). ANOVA tests were used to identify significant differences between the means of the three study groups. Scheffé's tests were used to determine variation in group's means. Finally, paired t-tests assessed the significance of changes within each group between time periods 0-4, 4-8 and 0-8 weeks. The data for each parameter from each subject were added, averaged and weighted depending on the number of teeth used from the subject.

Chapter 4

Results

Intra-examiner error

Repeat examinations were carried out on 5 subjects between the ages of 26-63 years measuring GI, PBI, PSS, PD and PAL at 4 sites around each tooth (1 day between examinations) to establish intra-examiner error. The same examination procedures were used as in the subsequent study. The accuracy of reproducibility of measurements is presented in Table 4.1. Accuracy of 100% agreement and agreement to 1 unit are calculated.

Table 4.1 Cross tabulations for GI, PSS, PBI, PD and PAL

Data in bold indicate 100% accuracy for each score for an index or measurement parameter

4.1a GI cross tabulations

G1/G2	0	1	2	3	total
0	**46**	9	1	0	56
1	4	**39**	4	0	47
2	2	3	**6**	2	13
3	0	0	1	**5**	6
total	52	51	12	7	122

100% Accuracy	Accuracy to 1 unit
96/122 = 78.69%	119/122 = 97.54%

4.1b PSS cross tabulations

P1/P2	0	1	2	3	4	5	total
0	**64**	4	2	0	0	0	70
1	5	**25**	1	0	0	0	31
2	0	2	**6**	0	0	0	8
3	0	1	2	**7**	0	0	10
4	0	0	0	1	**1**	0	2
5	0	0	0	0	0	**1**	1
total	69	32	11	8	1	1	122

100% Accuracy	Accuracy to 1 unit
104/122 = 86.07%	119/122 = 97.54%

4.1c PBI cross tabulations

B1/B2	0	1	2	3	4	total
0	**68**	5	2	0	0	75
1	5	**13**	2	0	0	20
2	1	2	**5**	1	0	9
3	0	3	2	**2**	0	7
4	0	0	1	0	**0**	1
total	74	23	12	3	0	112

100% Accuracy	Accuracy to 1 unit
88/112 = 79.46%	105/112 = 93.75%

4.1d PD cross tabulations

P1/P2	0	1	2	3	4	5	6	7	8	9	10	11	total
0	**33**	4	0	0	0	0	0	0	0	0	0	0	37
1	10	**73**	8	0	0	0	0	0	0	0	0	0	91
2	0	8	**60**	5	1	0	0	0	0	0	0	0	74
3	0	0	8	**21**	5	0	0	0	0	0	0	0	34
4	0	0	2	4	**13**	2	1	0	0	0	0	0	22
5	0	0	0	0	1	**5**	1	0	0	0	0	0	7
6	0	0	0	0	2	2	**6**	1	0	0	0	0	11
7	0	0	0	0	0	0	0	**0**	1	0	1	0	2
8	0	0	0	0	0	1	0	1	**1**	0	2	0	5
9	0	0	0	0	0	0	1	0	0	**0**	0	0	1
10	0	0	0	0	0	0	0	0	0	0	**0**	1	1
11	0	0	0	0	0	0	0	0	0	1	0	**2**	3
total	43	85	78	30	22	10	9	2	2	1	3	3	288

100% Accuracy	Accuracy to 1 unit
214/288 = 74.31%	276/288 = 95.83%

4.1e PAL cross tabulations

PL1/2	0	1	2	3	4	5	6	7	8	9	10	11	total
0	**20**	2	1	0	0	0	0	0	0	0	0	0	23
1	7	**30**	6	0	0	0	0	0	0	0	0	0	43
2	6	6	**49**	7	1	0	0	0	0	0	0	0	69
3	0	0	7	**25**	6	0	0	0	0	0	0	0	38
4	0	0	1	9	**26**	6	3	0	0	0	0	0	45
5	0	0	0	1	2	**12**	2	0	0	0	0	0	17
6	0	0	0	0	3	3	**10**	2	2	0	0	0	20
7	0	0	0	0	0	0	0	**7**	1	0	0	0	8
8	0	0	0	0	0	0	1	2	**10**	0	0	0	13
9	0	0	0	0	0	0	0	1	0	**0**	1	0	2
10	0	0	0	0	0	0	0	0	0	1	**1**	0	2
11	0	0	0	0	0	0	0	0	0	0	1	**7**	8
Total	33	38	64	42	38	21	16	12	13	1	3	7	288

100% Accuracy	Accuracy to 1 unit
197/288 = 68.40%	268/288 = 93.06%

Profile of the study population

Fifty-eight subjects were recruited, but due to several dropouts during the study, data from 9 subjects were not used in the final analyses. The 49 subjects consisted of 25 males and 24 females. Table 4.2 shows the age and gender distribution; there were no statistically significant age or gender differences between the groups (Pearson Chi-square, $p = 0.772$ and $p = 0.889$ respectively).

Table 4.2 Distribution in treatment groups of subjects by age and gender

Group	Female	Male	N	Mean age	SD	95% CI	95% CI	Min age	Max age
PLB	7	8	15	46.53	7.90	42.16	50.91	32	61
CHX	10	8	18	44.89	11.62	39.11	50.67	26	63
TTO	7	9	16	45.50	8.84	40.79	50.21	30	62
Total	24	25	49	45.59	9.53	42.85	48.33	26	63

Analyses of data from all teeth

In the following tables, P values in bold indicate statistical significance and P values preceded by "^" indicate statistical tests nearing statistical significance.

Table 4.3 ANOVA tests for data relating to all teeth

Parameter	P value
GI average, week 0	0.433
GI average, week 4	0.890
GI average, week 8	0.749
PSS average, week 0	0.605
PSS average, week 4	0.169
PSS average, week 8	0.300
PBI average, week 0	0.498
PBI average, week 4	0.663
PBI average, week 8	0.921
GI average change, weeks 0-4	0.648
GI average change, weeks 0-8	0.670
GI average change, weeks 4-8	0.442
PSS average change, weeks 0-4	0.401
PSS average change, weeks 0-8	0.425
PSS average change, weeks 4-8	0.911
PBI average change, weeks 0-4	0.085
PBI average change, weeks 0-8	0.640
PBI average change, weeks 4-8	0.552
PD average, week 0	**0.002**
PD average, week 4	**0.004**
PD average, week 8	**0.002**
PAL average, week 0	**0.024**
PAL average, week 4	**0.047**
PAL average, week 8	0.026
PD average change, weeks 0-4	0.783
PD average change, weeks 0-8	0.283
PD average change, weeks 4-8	0.783
PAL average change, weeks 0-4	0.601
PAL average change, weeks 0-8	0.307
PAL average change, weeks 4-8	0.330

Statistically significant tests of inter-group variations are presented in the following sections. Non-significant data or data that do not show any trends have not been shown.

Table 4.4 Descriptive statistics for PD averages

Week	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0	PLB	3.61	1.56	15	3.12	4.09	**0.002**
	CHX	3.65	1.96	18	3.21	4.07	
	TTO	4.63	3.71	16	4.20	5.05	
4	PLB	3.44	1.64	15	2.92	3.94	**0.004**
	CHX	3.55	2.00	18	3.10	3.99	
	TTO	4.47	3.91	49	4.02	4.91	
8	PLB	3.33	1.64	15	2.85	3.81	**0.002**
	CHX	3.59	2.00	18	3.16	4.00	
	TTO	4.45	3.91	16	4.03	4.87	

Scheffé's tests

	Week 0			Week 4			Week 8		
Group	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**
PLB	-	0.994	**0.010**	-	0.944	**0.014**	-	0.729	**0.004**
CHX	0.994	-	**0.008**	0.944	-	**0.020**	0.729	-	**0.020**
TTO	**0.010**	**0.008**	-	**0.014**	**0.020**	-	**0.004**	**0.020**	-

PD average, week 0: The PLB and CHX groups were significantly different to the TTO group but not significantly different to each other. From the outset of the study, the TTO group had a significantly greater PD mean than the PLB and CHX groups.

PD average, week 4: The PLB and CHX groups were significantly different to TTO, but not significantly different to each other. All groups showed a decrease in mean PD. TTO continued to have a greater PD mean than the PLB and CHX groups at week 4.

PD average, week 8: The PLB and CHX groups were significantly different to TTO, but not significantly different to each other. In comparison to week 4, the PLB and TTO groups showed a decrease in the mean PD whereas the CHX group showed an increase.

Table 4.5 Descriptive statistics for PAL averages.

Week	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0	PLB	4.07	2.60	15	3.44	4.70	**0.024**
	CHX	4.27	2.83	18	3.71	4.82	
	TTO	5.15	4.34	16	4.59	5.70	
4	PLB	3.98	2.66	15	3.33	4.62	**0.047**
	CHX	4.25	3.02	18	3.68	4.81	
	TTO	5.00	4.34	16	4.43	5.56	
8	PLB	3.87	2.77	15	3.22	4.50	**0.026**
	CHX	4.29	3.24	18	3.72	4.85	
	TTO	5.03	4.04	16	4.46	5.58	

Scheffé's tests

	Week 0			Week 4			Week 8		
Group	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**
PLB	-	0.897	**0.044**	-	0.819	^0.066	-	0.614	**0.031**
CHX	0.994	-	^0.087	0.819	-	0.178	0.614	-	0.187
TTO	**0.044**	^0.087	-	^0.066	0.178	-	**0.031**	0.187	-

PAL average, week 0: The PLB group was significantly different to TTO. However the PLB and CHX groups, and CHX and TTO were not significantly different to each other. The Scheffé's test indicated that the differences between the CHX and TTO means were approaching significance (0.087).

PAL average, week 4: There were significant differences between the treatment groups. The PAL means of all groups decreased over the 4 week period and the Scheffé's test indicated that the differences between the PLB and TTO group means were approaching significance (0.066)..

PAL average, week 8: The PLB group was significantly different to the TTO group (smaller PAL). However the PLB and CHX groups and CHX and TTO groups were not significantly different to each other. PLB showed decrease in mean PAL at week 8

compared to week 4, but an increase for the CHX and TTO groups. These changes were extremely small.

Analysis of parameters for anterior and posterior teeth

To test whether clinical changes occurred more frequently in parameters related to the anterior than the posterior teeth data relating to the anterior and posterior teeth were analysed separately.

Table 4.6 ANOVA tests for anterior and posterior teeth

Parameter	Anterior teeth	Posterior teeth
GI average, week 0	0.240	0.645
GI average, week 4	0.572	0.949
GI average, week 8	0.394	0.845
PSS average, week 0	0.655	0.616
PSS average, week 4	0.208	0.161
PSS average, week 8	0.131	0.659
PBI average, week 0	0.236	0.808
PBI average, week 4	0.794	0.486
PBI average, week 8	0.928	0.430
GI average change, weeks 0-4	0.715	0.340
GI average change, weeks 0-8	0.500	0.447
GI average change, weeks 4-8	0.809	0.339
PSS average change, weeks 0-4	0.438	0.569
PSS average change, weeks 0-8	0.303	0.670
PSS average change, weeks 4-8	0.797	0.790
PBI average change, weeks 0-4	0.356	**0.041**
PBI average change, weeks 0-8	0.202	^0.057
PBI average change, weeks 4-8	0.762	0.353
PD average, week 0	**0.001**	0.094
PD average, week 4	**0.003**	^0.068
PD average, week 8	**0.001**	^0.055
PAL average, week 0	**0.002**	0.535
PAL average, week 4	**0.010**	0.493
PAL average, week 8	**0.005**	0.349
PD average change, weeks 0-4	0.819	0.555
PD average change, weeks 0-8	0.074	0.736
PD average change, weeks 4-8	**0.038**	0.959
PAL average change, weeks 0-4	0.410	0.725
PAL average change, weeks 0-8	**0.038**	0.737
PAL average change, weeks 4-8	**0.001**	0.500

The differences in PD and PAL between the groups (anterior teeth) were present from the beginning of the study. The PAL averages for weeks 4 and 8 showed that the TTO and PLB groups remained significantly different. However the CHX group's mean began to approach that of the TTO group and eventually was not significantly different (see page 60 for Scheffé test relating to PAL at weeks 4 and 8).

The placebo group had the greatest changes in PD during weeks 4-8 (mean change 0.37 mm), PAL average changes during weeks 0-8 (mean change 0.25 mm) and 4-8 (mean change 0.23 mm). The PBI average changes for weeks 0-4 and 0-8 became or approached significant differences in posterior teeth. The TTO group in both instances had the greatest change (week 0-4 mean = 0.56, week 0-8 mean = 0.50)

Data relating to posterior teeth

The following tables consist of statistically significant tests or data which approached statistical significance.

Table 4.7 Descriptive statistics for PBI average change, posterior teeth.

Weeks	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	0.25	1.34	15	-0.04	0.54	**0.041**
	CHX	0.14	0.71	18	-0.01	0.38	
	TTO	0.57	1.11	16	0.33	0.80	
4-8	PLB	-0.32	1.29	15	-0.07	0.06	0.353
	CHX	0.04	1.27	18	-0.02	0.37	
	TTO	-0.06	1.60	16	-0.03	0.23	
0-8	PLB	-0.07	1.18	15	-0.04	0.30	^0.057
	CHX	0.18	1.36	18	-0.01	0.49	
	TTO	0.50	1.22	16	0.20	0.79	

Scheffé's tests

	Week 0		
Group	**PLB**	**CHX**	**TTO**
PLB	-	0.845	0.249
CHX	0.845	-	^0.051
TTO	0.249	^0.051	-

PBI average change for weeks 0-4, posterior teeth: The TTO group had the largest statistically significant change in PBI for weeks 0-4. The Scheffé's tests showed that the CHX group's change was nearing significant difference to the TTO group. However PLB and CHX and, CHX and TTO were not significantly different to each other between weeks 0-4.

PBI average change for weeks 4-8, posterior teeth: No statistically significant changes were seen between the groups for weeks 4-8. The PLB group showed an increase in PBI

PBI average change for weeks 0-8, posterior teeth: No statistically significant changes were seen between the groups for weeks 0-8. The TTO group had the largest change in PBI for week 0-8. However, the differences between the groups between weeks 0-8 approached statistical significance ($p = 0.057$)

Data relating to anterior teeth

The following results consist of statistically significant tests or data which approached statistical significance.

Table 4.8 Descriptive statistics for PD averages, anterior teeth

Week	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0	PLB	3.30	1.22	13	2.74	3.85	**0.001**
	CHX	3.33	1.49	15	2.82	3.83	
	TTO	4.69	3.19	14	4.14	5.23	
4	PLB	3.19	1.44	13	2.59	3.78	**0.003**
	CHX	3.24	1.58	15	2.69	3.78	
	TTO	4.51	3.41	14	3.92	5.09	
8	PLB	2.98	1.64	13	2.40	3.54	**0.001**
	CHX	3.32	2.00	15	2.80	3.83	
	TTO	4.45	3.91	14	3.88	5.00	

Scheffé's tests

	Week 0			Week 4			Week 8		
Group	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**
PLB	-	0.997	**0.004**	-	0.992	**0.011**	-	0.672	**0.003**
CHX	0.997	-	**0.003**	0.922	-	**0.010**	0.672	-	**0.018**
TTO	**0.004**	**0.003**	-	**0.011**	**0.010**	-	**0.003**	**0.018**	-

PD average for week 0, anterior teeth: The PLB and CHX groups were significantly different to TTO. However, the PLB and CHX groups were not significantly different to each other.

PD average for week 4, anterior teeth: The PLB and CHX groups were significantly different to TTO. However, the PLB and CHX groups were not significantly different to each other. The relationship between the PLB and CHX groups and TTO was maintained over the first 4-week period. All groups showed a small decrease in PD mean over the 4 week period.

PD average for week 8, anterior teeth: The PLB and CHX groups were significantly different to TTO. However, the PLB and CHX groups were not significantly different to each other at week 8, again maintaining the baseline relationship. PLB and TTO groups showed a decrease in PD over the 4-8 week period, but CHX increased in PD over the same period.

Table 4.9 Descriptive statistics for PAL averages, the anterior teeth

Week	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0	PLB	3.71	2.23	13	2.99	4.41	**0.002**
	CHX	4.09	2.12	15	3.44	4.73	
	TTO	5.52	3.66	14	4.81	6.21	
4	PLB	3.69	2.50	13	2.93	4.44	**0.010**
	CHX	4.12	2.60	15	3.43	4.80	
	TTO	5.31	3.57	14	4.57	6.05	
8	PLB	3.46	2.41	13	2.69	4.22	**0.005**
	CHX	4.29	2.82	15	3.59	4.98	
	TTO	5.32	3.56	14	4.56	6.06	

Scheffé's tests

	Week 0			Week 4			Week 8		
Group	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**
PLB	-	0.725	**0.003**	-	0.700	**0.013**	-	0.278	**0.005**
CHX	0.725	-	**0.016**	0.700	-	0.069	0.278	-	0.140
TTO	**0.003**	**0.016**	-	**0.013**	0.069	-	**0.005**	0.140	-

PAL average for week 0, anterior teeth: The PLB and CHX groups were significantly different to TTO. However the PLB and CHX groups were not significantly different to each other. This follows the relationship seen in PD for each group.

PAL average for week 4, anterior teeth: The PLB group was significantly different to TTO. However the PLB and CHX groups and the CHX and TTO groups were not significantly different to each other. The PLB and TTO groups had decreased PAL between weeks 0-4, however CHX increased over the same period.

PAL average for week 8, anterior teeth: The PLB group was significantly different to TTO. However the CHX group was not significantly different to TTO. The PLB group continued to exhibit small decreases in PAL between weeks 4-8, however the CHX and TTO group's PAL increased (albeit very small for TTO) over the same period.

Table 4.10 Descriptive statistics for PD average change, anterior teeth.

Week	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	0.11	0.77	13	-0.10	0.33	0.819
	CHX	0.09	0.91	15	-0.11	0.29	
	TTO	0.18	0.87	14	-0.03	0.39	
4-8	PLB	0.21	0.63	13	0.05	0.37	**0.038**
	CHX	-0.08	0.57	15	-0.22	0.07	
	TTO	0.06	0.69	14	-0.09	0.22	
0-8	PLB	0.33	0.69	13	0.11	0.53	^0.074
	CHX	0.01	1.01	15	-0.17	0.20	
	TTO	0.24	0.66	14	0.03	0.44	

Scheffé's tests

	Week 0			Week 4			Week 8		
Group	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**
PLB	-	0.998	0.908	-	**0.038**	0.416	-	0.092	0.844
CHX	0.998	-	0.828	**0.038**	-	0.442	0.092	-	0.264
TTO	0.908	0.828	-	0.416	0.442	-	0.844	0.264	-

PD average change between weeks 0-4, anterior teeth: No significant differences occurred in this time period however the TTO group had the greatest change in PD.

PD average change between weeks 4-8, anterior teeth: There was a significant difference between changes in PD between weeks 4-8. The PLB and CHX groups were significantly different to each other. The PLB group had the greatest change in PD.

PD average change between weeks 0-8, anterior teeth: no significant differences occurred in this time period. The PLB group had the greatest change in PD.

Table 4.11 Descriptive statistics for PAL average change, anterior teeth.

Weeks	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	0.02	0.74	13	-0.25	0.28	0.410
	CHX	-0.03	1.31	15	-0.27	0.21	
	TTO	0.20	1.00	14	-0.06	0.47	
4-8	PLB	0.23	0.63	13	0.08	0.38	**0.001**
	CHX	-0.17	0.60	15	-0.30	-0.33	
	TTO	-0.04	0.52	14	-0.15	0.14	
0-8	PLB	0.25	0.64	13	-0.03	0.53	**0.038**
	CHX	-0.20	1.50	15	-0.45	0.05	
	TTO	0.20	0.20	14	-0.07	0.47	

Scheffé's tests

	Week 0			Week 4			Week 8		
Group	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**
PLB	-	0.968	0.614	-	**0.001**	0.087	-	**0.068**	0.967
CHX	0.968	-	0.439	**0.001**	-	0.261	**0.068**	-	0.114
TTO	0.614	0.439	-	0.087	0.261	-	0.967	0.114	-

PAL average change between weeks 0-4, anterior teeth: no significant differences occurred in this time period; however, the TTO group had the greatest change in PAL.

PAL average change in PAL between weeks 4-8, anterior teeth: There was a significant difference in PAL between weeks 4-8. The Scheffé tests showed that the differences between the means of the PLB and CHX groups were statistically significant. The PLB group had the greatest change in PAL.

PAL average change between weeks 0-8, anterior teeth: There was a significant difference in PD between weeks 0-8. The Scheffé tests showed that the differences between the means of the PLB and CHX groups were approaching statistical significance. The PLB group had the greatest change in PAL.

Analysis of data considering pocket depth

In sites where the pocket depth was ≥ 5 mm, the ability of topically applied agents to have clinical effects diminishes due to their inability to penetrate into the pocket. This results in a failure to provide a minimum concentration required for therapeutic activity. Consequently, data for pockets depth (< 5mm and ≥ 5mm) were separated and analysed to assess if TTO (unlike CHX) affects deep sites.

The significant differences between the groups was in PD and PAL at week 0, confirming that the groups were significantly different with respect to these parameters at the start of the study. PBI average changes became significant for weeks 0-4 and 0-8 in the posterior teeth with deep sites (Table 4.12). The Scheffé tests showed that the TTO group exhibited the greatest change for PBI compared to the placebo and CHX groups. PBI average changes in posterior teeth with shallow pockets approached significance (PBI average changes between weeks 0-4 $p = 0.062$ and weeks 0-8 $p = 0.075$), the TTO group exhibited the greatest change of all groups.

Table 4.12 ANOVA tests for anterior and posterior teeth grouped by initial pocket depth

	Anterior teeth		Posterior teeth	
Parameter	**< 5mm**	**≥ 5mm**	**< 5mm**	**≥ 5mm**
GI average for week 0	0.242	0.180	0.747	0.556
GI average for week 4	0.711	0.405	0.909	0.961
GI average for week 8	0.530	0.287	0.873	0.841
PSS average for week 0	0.625	0.971	0.680	0.759
PSS average for week 4	0.210	0.207	0.167	0.167
PSS average for week 8	0.155	0.174	0.706	0.650
PBI average for week 0	0.229	0.219	0.846	0.589
PBI average for week 4	0.797	0.702	0.501	0.595
PBI average for week 8	0.892	0.900	0.432	0.457
GI average change between weeks 0-4	0.716	0.766	0.371	0.415
GI average change between weeks 0-8	0.433	0.431	0.444	0.431
GI average change between weeks 4-8	0.695	0.759	0.326	0.379
PSS average change between weeks 0-4	0.289	0.341	0.533	0.507
PSS average change between weeks 0-8	0.255	0.226	0.726	0.654
PSS average change between weeks 4-8	0.844	0.820	0.794	0.730
PBI average change between weeks 0-4	0.290	0.241	0.062	**0.039**
PBI average change between weeks 0-8	0.230	0.182	0.075	**0.020**
PBI average change between weeks 4-8	0.683	0.572	0.312	0.310
PD average for week 0	0.493	**0.001**	0.301	0.072
PD average for week 4	0.623	**0.006**	0.517	0.098
PD average for week 8	0.552	**0.001**	0.291	0.146
PAL average for week 0	0.630	**0.002**	0.253	0.631
PAL average for week 4	0.511	**0.014**	0.667	0.444
PAL average for week 8	0.165	**0.003**	0.482	0.598
PD average change between weeks 0-4	0.984	0.624	0.581	0.680
PD average change between weeks 0-8	0.204	0.109	0.596	0.814
PD average change between weeks 4-8	0.150	0.511	0.870	0.825
PAL average change between weeks 0-4	0.765	0.751	0.456	0.476
PAL average change between weeks 0-8	0.113	0.100	0.659	0.596
PAL average change between weeks 4-8	**0.042**	0.213	0.358	0.565

Descriptive statistics of posterior teeth with ≥ 5mm pocket depth

Table 4.13 Descriptive statistics for PBI average change, posterior teeth (PD ≥ 5mm).

Weeks	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	0.21	1.33	14	-0.09	0.51	**0.039**
	CHX	0.13	0.68	18	-0.12	0.39	
	TTO	0.57	1.09	16	0.33	0.80	
4-8	PLB	-0.34	1.32	14	-0.72	0.04	0.310
	CHX	0.03	1.17	18	-0.30	0.35	
	TTO	-0.02	1.51	16	-0.31	0.28	
0-8	PLB	-0.13	1.48	14	-0.50	0.25	**0.020**
	CHX	0.16	1.31	18	-0.16	0.48	
	TTO	0.55	1.11	16	0.25	0.84	

Scheffé's tests

	Week 0			Week 4			Week 8		
Group	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**
PLB	-	0.927	0.188	-	0.362	0.424	-	0.513	**0.024**
CHX	0.927	-	0.056	0.362	-	0.982	0.513	-	0.214
TTO	0.188	0.056	-	0.424	0.982	-	**0.024**	0.214	-

PBI average changes between weeks 0-4, posterior teeth with deep pockets:

Average change in PBI was greatest for the TTO group for weeks 0-4. This was statistically significant.

PBI average changes between weeks 4-8, posterior teeth with deep pockets: The PLB group had the greatest negative change during time period 4-8, whereas little change was seen in the other groups. Between weeks 0-4, the average change for the TTO group approached statistical significance when compared to the average change for the CHX group.

PBI average changes between weeks 0-8, posterior teeth with deep pockets:

Over the length of the trial (weeks 0-8) the TTO group average changes became significantly different to the PLB group.

Descriptive statistics for posterior teeth with < 5 mm pocket depth

Table 4.14 Descriptive statistics for PBI change, posterior teeth (PD < 5mm).

Weeks	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	0.25	1.34	14	-0.03	0.53	^0.062
	CHX	0.14	0.71	18	-0.11	0.38	
	TTO	0.54	0.99	16	0.30	0.77	
4-8	PLB	-0.32	1.29	14	-0.70	0.05	0.312
	CHX	0.05	1.24	18	-0.27	0.37	
	TTO	-0.06	1.50	16	-0.37	0.25	
0-8	PLB	-0.08	1.48	14	-0.45	0.29	^0.075
	CHX	0.19	1.32	18	-0.13	0.51	
	TTO	0.48	1.21	16	0.17	0.79	

Scheffé's tests

	Week 0			Week 4			Week 8		
Group	PLB	CHX	TTO	PLB	CHX	TTO	PLB	CHX	TTO
PLB	-	0.842	0.296	-	0.322	0.549	-	0.561	0.079
CHX	0.842	-	0.073	0.322	-	0.889	0.561	-	0.427
TTO	0.296	0.073	-	0.549	0.889	-	0.079	0.427	-

PBI average change between weeks 0-4, posterior teeth with shallow pockets: All groups exhibited positive changes in the first 4 weeks, with the TTO group exhibiting the largest change. This change approached statistical significance.

PBI average change between weeks 4-8, posterior teeth with shallow pockets:

During the 4-8 week period the PLB exhibited the greatest change however this was an increase in PBI.

PBI average change between weeks 0-8, posterior teeth with shallow pockets:

The overall positive change was greatest in the TTO group, and this also approached statistical significance. These results reflected the changes seen in deep sites.

Data from anterior teeth with pockets ≥ 5mm

Table 4.15 Descriptive statistics for PD, anterior teeth (PD ≥ 5mm).

Week	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0	PLB	5.32	0.96	13	4.91	5.73	**0.001**
	CHX	5.46	0.85	14	5.09	5.82	
	TTO	6.30	2.05	14	5.94	6.63	
4	PLB	4.65	1.35	13	4.08	5.22	**0.006**
	CHX	4.98	1.53	14	4.46	5.49	
	TTO	5.87	2.71	14	5.36	6.37	
8	PLB	4.50	1.61	13	3.97	5.02	**0.001**
	CHX	5.06	1.57	14	4.58	5.53	
	TTO	5.88	2.19	14	5.42	6.34	

Scheffé's tests

	Week 0			Week 4			Week 8		
Group	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**
PLB	-	0.882	**0.003**	-	0.689	**0.010**	-	0.292	**0.001**
CHX	0.882	-	**0.008**	0.689	-	0.056	0.292	-	^0.053
TTO	**0.003**	**0.008**	-	**0.010**	0.056	-	**0.001**	^0.053	-

PD averages for week 0, anterior teeth with deep pockets:

The PD of the TTO group was significantly greater than the other groups, this relation was maintained throughout the 8 weeks between the TTO and PLB groups.

PD averages for week 4, anterior teeth with deep pockets:

The PD of the TTO group was significantly greater than the other groups, the significant difference between the TTO and CHX groups was lost but the P value (Scheffé's test) was close to becoming statistically significant.

PD averages for week 8, anterior teeth with deep pockets: The PD of the TTO group was significantly different to the other groups (larger), the significant difference

between the TTO and CHX groups was lost but the P value (Scheffé's test) was close to becoming statistically significant.

Table 4.16 Descriptive statistics for PAL, anterior teeth (PD ≥ 5mm).

Week	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0	PLB	5.72	2.32	13	5.11	6.31	**0.002**
	CHX	5.85	1.31	14	5.30	6.39	
	TTO	7.07	2.44	14	6.54	7.60	
4	PLB	5.17	2.49	13	4.42	5.92	**0.014**
	CHX	5.54	2.40	14	4.85	6.21	
	TTO	6.61	2.86	14	5.92	7.27	
8	PLB	4.93	2.58	13	4.18	5.67	**0.003**
	CHX	5.75	2.45	14	5.07	6.41	
	TTO	6.70	2.67	14	6.04	7.35	

Scheffé's tests

	Week 0			Week 4			Week 8		
Group	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**
PLB	-	0.948	**0.006**	-	0.768	**0.022**	-	0.270	**0.004**
CHX	0.948	-	**0.009**	0.768	-	^0.084	0.270	-	0.134
TTO	**0.006**	**0.009**	-	**0.022**	^0.084	-	**0.004**	0.134	-

PAL averages for week 0, anterior teeth with deep pockets:

The TTO group's PAL was significantly different (ANOVA) to the other groups (larger), this relation was maintained throughout the 8 weeks between the TTO and PLB groups.

PAL averages for week 4, anterior teeth with deep pockets:

The PAL of the TTO group was significantly different to the other groups (larger), the significant difference between the TTO and CHX groups was lost but the P value (Scheffé's test) was close to becoming statistically significant.

PAL averages for week 8, anterior teeth with deep pockets: The PAL of the TTO group was significantly different to the other groups (larger), the significant difference between the TTO and CHX groups was lost.

Data from buccal and lingual surfaces

The intra-oral distribution of oral hygiene products can vary greatly, with buccal surfaces receiving the majority of product. An analysis comparing buccal/labial and palatal/lingual surface responses was carried out to determine if such an effect had occurred.

Table 4.17 ANOVA test for data from buccal and lingual surfaces

Parameter	Buccal surfaces	Lingual surfaces
GI average for week 0	0.681	0.344
GI average for week 4	0.999	0.661
GI average for week 8	0.917	0.648
PSS average for week 0	0.521	0.663
PSS average for week 4	0.291	0.261
PSS average for week 8	0.406	0.263
PBI average for week 0	0.082	0.957
PBI average for week 4	0.812	0.537
PBI average for week 8	0.854	0.977
GI average change between weeks 0-4	0.541	0.886
GI average change between weeks 0-8	0.714	0.456
GI average change between weeks 4-8	0.676	0.270
PSS average change between weeks 0-4	0.654	0.208
PSS average change between weeks 0-8	0.899	0.155
PSS average change between weeks 4-8	0.852	0.361
PBI average change between weeks 0-4	**0.021**	0.469
PBI average change between weeks 0-8	0.254	0.990
PBI average change between weeks 4-8	0.789	0.486
PD average for week 0	0.164	^0.055
PD average for week 4	0.250	^0.056
PD average for week 8	0.157	**0.015**
PAL average for week 0	0.401	0.196
PAL average for week 4	0.653	0.204
PAL average for week 8	0.355	0.121
PD average change between weeks 0-4	0.869	0.890
PD average change between weeks 0-8	0.841	0.749
PD average change between weeks 4-8	0.660	0.666
PAL average change between weeks 0-4	0.505	0.907
PAL average change between weeks 0-8	0.525	0.846
PAL average change between weeks 4-8	0.297	0.833

Data from lingual surfaces

Table 4.18 Descriptive statistics for PD averages, palatal/lingual surfaces.

Week	Group	n	Mean	SD	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0	PLB	15	2.71	2.52	2.01	3.40	^0.055
	CHX	18	3.02	3.43	2.40	3.63	
	TTO	16	3.79	4.73	3.18	4.40	
4	PLB	15	2.67	2.57	1.96	3.37	^0.056
	CHX	18	2.90	3.44	2.27	3.51	
	TTO	16	3.73	4.82	3.11	4.34	
8	PLB	15	2.57	2.18	1.93	3.21	**0.015**
	CHX	18	2.88	3.48	2.32	3.44	
	TTO	16	3.78	4.15	3.21	4.33	

Scheffé tests for PD

	Week 0			Week 4			Week 8		
Group	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**	**PLB**	**CHX**	**TTO**
PLB	-	0.798	0.073	-	0.884	0.084	-	0.761	**0.024**
CHX	0.798	-	0.210	0.884	-	0.172	0.761	-	0.089
TTO	0.073	0.210	-	0.084	0.172	-	**0.024**	0.089	-

PD averages for week 0, lingual surfaces: The PD of the TTO group was different (ANOVA) to the other groups (larger), however his was not statistically significant. The P value (ANOVA) was close to becoming statistically significant.

PD averages for week 4, lingual surfaces: The PD of the TTO group was different to the other groups (larger), however his was not statistically significant. The P value (ANOVA) was close to becoming statistically significant.

PD averages for week 8, lingual surfaces: The PD of the TTO group was significantly greater than that of the other groups, the significant difference was between the TTO and PLB groups (Scheffé's test). The TTO group's PD remained relatively stable, but the PD of the PLB changed slightly. This statistically significant result was probably because:

- the PD of the TTO and PLB groups was different at week 0;
- a small fluctuation in the PLB group but not the TTO caused the means of the two groups to vary enough to register as statistically significant.

Data from buccal surfaces

Table 4.19 Descriptive statistics for PBI averages, buccal surfaces.

Week	Group	n	Mean	SD	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0	PLB	15	2.36	2.00	1.98	2.72	^0.082
	CHX	18	2.49	2.07	2.16	2.82	
	TTO	16	2.89	1.82	2.56	3.21	
4	PLB	15	2.03	2.45	1.53	2.51	0.812
	CHX	18	2.24	2.65	1.80	2.67	
	TTO	16	2.13	2.69	1.70	2.56	
8	PLB	15	2.08	1.80	1.58	2.58	0.854
	CHX	18	2.22	2.94	1.78	2.65	
	TTO	16	2.26	2.90	1.82	2.70	

PBI average for week 0 on buccal sites: The TTO group had the highest PBI at week 0. This parameter approached statistical significance.

PBI average for week 4 on buccal sites: The CHX group had the highest PBI for week 4 but did not reach statistical significance.

PBI average for week 8 on buccal sites: There were no statistically significant differences between the groups for week 8.

Table 4.20 Descriptive statistics for PBI average change, buccal/labial surfaces.

Weeks	Group	n	Mean	SD	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	15	0.33	1.92	0.03	0.62	**0.021**
	CHX	18	0.26	1.37	-0.03	0.51	
	TTO	16	0.75	1.39	0.49	1.00	
4-8	PLB	15	-0.05	2.40	-0.40	0.29	0.789
	CHX	18	0.01	1.76	-0.28	0.32	
	TTO	16	-0.13	1.25	-0.43	0.17	
0-8	PLB	15	0.27	2.22	-0.10	0.65	0.254
	CHX	18	0.27	2.07	-0.05	0.61	
	TTO	16	0.62	1.72	0.28	0.95	

Scheffé test

	Week 0-4		
Group	**PLB**	**CHX**	**TTO**
PLB	-	0.934	0.106
CHX	0.934	-	**0.032**
TTO	0.106	**0.032**	-

PBI average change between weeks 0-4, buccal surfaces: The TTO group had the largest change during weeks 0-4. This reached statistical significance. The CHX group showed the smallest change in PBI for this time period. The Scheffé's test showed that the CHX and TTO groups were significantly different.

PBI average change between weeks 4-8, buccal surfaces: Very small changes occurred during this time period. The TTO group had the largest change during weeks 4-8 however this represented an increase in PBI rather than a decrease as in weeks 0-4. This did not reach statistical significance.

PBI average change between weeks 0-8, buccal surfaces: The TTO group showed the largest changes for weeks 0-8. This did not reach statistical significance. The PLB and CHX groups showed similar changes in PBI for this time period.

Analysis of PBI, GI and PSS data from all teeth to determine differences between groups in terms of improvement (lower scores in weeks 4 and 8 compared to baseline).

PBI data

Table 4.21 Descriptive statistics for sites with improvement in PBI, all teeth.

Weeks	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	38.30	15.51	15	29.71	46.89	**0.012**
	CHX	34.33	14.38	18	27.17	41.49	
	TTO	49.53	13.82	16	42.17	56.90	
4-8	PLB	28.93	18.17	15	18.87	38.99	0.572
	CHX	32.06	17.29	18	23.46	40.67	
	TTO	25.77	16.24	16	17.11	34.43	
0-8	PLB	40.79	21.27	15	29.01	52.57	0.563
	CHX	35.58	19.59	18	25.83	45.32	
	TTO	41.79	12.12	16	35.33	48.25	

Scheffé's tests

	Week 0-4		
Group	**PLB**	**CHX**	**TTO**
PLB	-	0.739	0.112
CHX	0.739	-	**0.015**
TTO	0.112	**0.015**	-

During weeks 0-4 and 0-8, the TTO group showed the most number of sites with improvement in PBI, the difference between the groups was statistically significant during weeks 0-4. The Scheffé's test showed that the TTO group was significantly different to the CHX group.

Table 4.22 Descriptive statistics for sites with improvement in PBI, anterior teeth.

Weeks	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	29.47	18.84	13	18.09	40.85	^0.067
	CHX	37.40	25.26	15	23.41	51.39	
	TTO	49.30	19.57	14	38.00	60.60	
4-8	PLB	31.35	21.54	13	18.33	44.36	0.655
	CHX	35.15	28.87	15	19.17	51.14	
	TTO	26.89	20.18	14	15.24	38.54	
0-8	PLB	35.11	22.86	13	18.33	44.36	0.855
	CHX	38.03	17.46	15	28.35	47.70	
	TTO	39.39	20.47	14	27.57	51.21	

During weeks 0-4 and 0-8, the TTO group showed the most number of sites with improvement in PBI, the difference between the groups (TTO and PLB) approached statistical significance during week 0-4. The CHX group showed the greatest number of sites with improvement during weeks 4-8.

Table 4.23 Descriptive statistics for sites with improvement in PBI, posterior teeth.

Weeks	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	38.70	16.35	15	29.64	47.75	**0.014**
	CHX	33.73	15.91	18	25.82	41.64	
	TTO	49.57	13.30	16	42.48	56.66	
4-8	PLB	22.47	18.09	15	12.45	32.48	0.517
	CHX	29.68	19.33	18	20.07	39.29	
	TTO	28.96	20.17	16	18.21	39.71	
0-8	PLB	31.66	19.25	15	20.99	42.32	0.208
	CHX	36.31	20.17	18	26.27	46.36	
	TTO	43.13	13.13	16	36.13	50.12	

Scheffé's test

	Week 0		
Group	**PLB**	**CHX**	**TTO**
PLB	-	0.650	0.152
CHX	0.650	-	**0.016**
TTO	0.152	**0.016**	-

During weeks 0-4 and 0-8, the TTO group showed the most number of sites with improvement in PBI, the difference between the groups approached statistical significance during weeks 0-4. Scheffé's test showed that the TTO group was significantly different to the CHX group which showed the greatest number of sites with improvement during weeks 4-8.

Table 4.24 Descriptive statistics for sites with improvement in PBI, buccal surfaces.

Weeks	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	38.74	20.13	15	27.59	49.89	**0.002**
	CHX	36.64	14.66	18	29.35	43.93	
	TTO	56.69	14.21	16	49.12	64.47	
4-8	PLB	29.96	24.05	15	16.65	43.28	0.826
	CHX	31.58	21.75	18	20.76	42.20	
	TTO	27.11	17.16	16	17.29	36.25	
0-8	PLB	41.50	22.38	15	29.10	53.89	0.382
	CHX	38.08	26.49	18	24.91	51.25	
	TTO	48.62	15.72	16	40.25	57.00	

Scheffé's tests

	Week 0-4		
Group	**PLB**	**CHX**	**TTO**
PLB	-	0.935	**0.015**
CHX	0.935	-	**0.004**
TTO	**0.015**	**0.004**	-

During weeks 0-4 and 0-8, the TTO group had the most number of sites with improvement in PBI, the difference between the groups approached statistical significance during week 0-4. The Scheffé's test showed that the TTO group was significantly different to the PLB and CHX groups. The CHX group showed the greatest number of sites with improvement during weeks 4-8. There were no statistically significant differences between the groups regarding improvement in PBI using data from lingual surfaces. In this data set results were varied; all groups had the

greatest number of sites with PBI improvement for the various time periods. The results for improvements in PBI showed several statistically significant results; indicating that the TTO group had a greater number sites with improvements than the other groups. This was consistent for most data sets.

GI data

Table 4.25 Descriptive statistics for sites with improvement in GI, all teeth.

Weeks	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	41.67	22.32	15	29.31	54.03	0.668
	CHX	39.80	20.87	18	29.42	50.18	
	TTO	46.62	24.46	16	33.59	59.65	
4-8	PLB	30.70	18.13	15	20.66	40.74	0.756
	CHX	27.89	20.35	18	17.77	38.01	
	TTO	25.68	17.03	16	16.60	34.75	
0-8	PLB	45.61	25.98	15	31.22	60.00	0.625
	CHX	53.06	19.74	18	43.24	62.88	
	TTO	46.62	26.81	16	32.34	60.91	

There were no statistically significant differences between the groups for the remaining data sets. The overall results for improvements in GI were variable however some possible trends were seen; the CHX group had the greatest number of time periods were it had the most improvements (7), next was the TTO group (6) and then the PLB group (5). Four out of the five 0-4 time periods showed that the TTO group had the greatest number of improved sites in GI. Overall the differences between the groups were not great, indicated by the lack of statistically significant results.

PSS data

Table 4.26 Descriptive statistics for sites with improvement in PSS, all teeth.

Weeks	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
0-4	PLB	29.39	24.07	15	16.05	42.72	0.343
	CHX	36.05	22.73	18	24.75	47.36	
	TTO	25.34	16.50	16	16.55	34.13	
4-8	PLB	31.14	26.79	15	16.31	45.97	0.427
	CHX	31.97	28.97	18	17.57	46.38	
	TTO	21.62	16.87	16	12.63	30.61	
0-8	PLB	34.65	28.33	15	18.96	50.34	0.597
	CHX	37.41	28.57	18	23.21	51.62	
	TTO	28.38	20.59	16	17.41	39.35	

There were no statistically significant differences between the groups; the CHX group had the most number of improved sites. The TTO group consistently had the lowest number of sites that improved for all time periods. Other data sets showed no statistically significant differences between groups. However, the CHX group generally had more improved sites in terms of PSS than the other groups. The TTO consistently showed fewer improved sites than the other groups. The CHX group had more time periods were it had the most improvements (11), next was the PLB group (4) and then the TTO group (1).

T-tests for all data sets

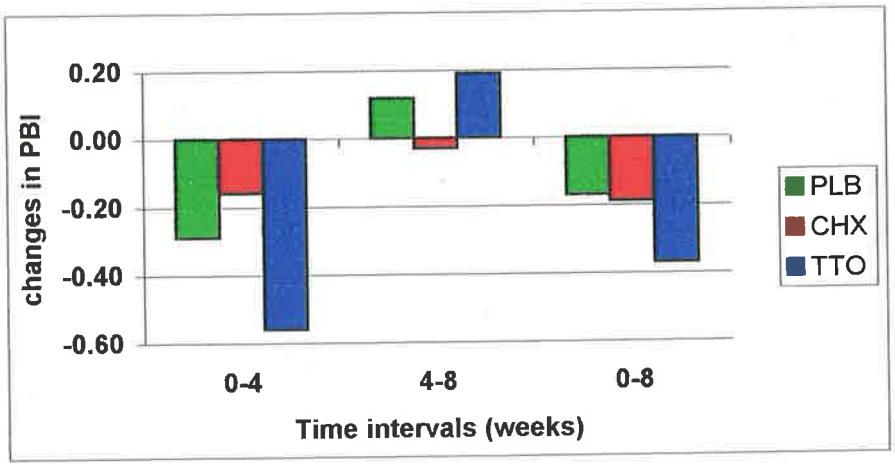
The following section is a summary of intra-group variations. Where significant changes were seen, figures accompany the tables.

T-tests for PBI

Table 4.27 Descriptive statistics for PBI, all teeth.

	PLB				CHX				TTO			
Time intervals (weeks)	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**
0 – 4	15	0.29	0.70	0.127	18	0.16	0.37	0.083	16	0.56	0.50	**0.000**
4 – 8	15	-0.12	0.76	0.555	18	0.03	0.56	0.818	16	-0.19	0.48	0.144
0 – 8	15	0.17	0.88	0.458	18	0.19	0.58	0.182	16	0.37	0.54	**0.014**

Figure 4.1 Average changes in PBI for weeks 0-4, 4-8 and 0-8.



All groups exhibited decreases in PBI for weeks 0-4. During this period, the TTO group had the largest decrease in PBI, this change was statistically significant. The CHX group had the smallest change in PBI during the same period. However during weeks 4-8 the TTO and PLB groups exhibited increases in PBI. Despite this increase in PBI, the TTO group showed the largest decrease between weeks 0-8. This change was statistically significant.

T-tests for PBI using data from posterior and anterior teeth

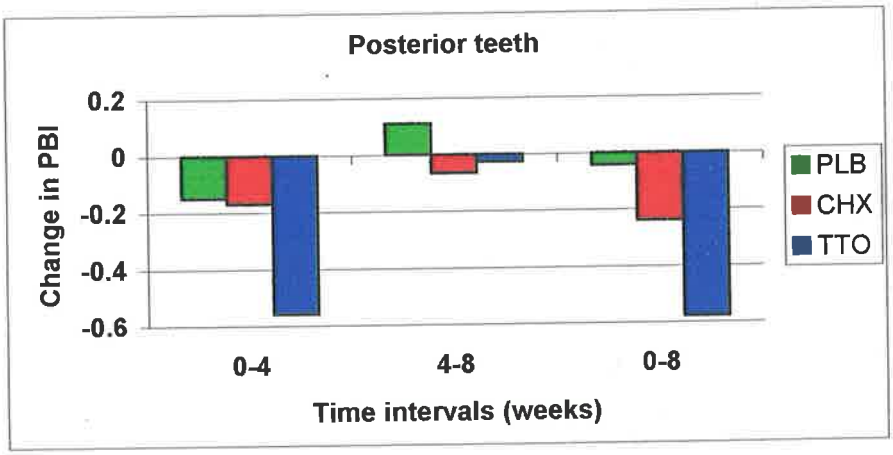
Table 4.28 Descriptive statistics for PBI, posterior teeth.

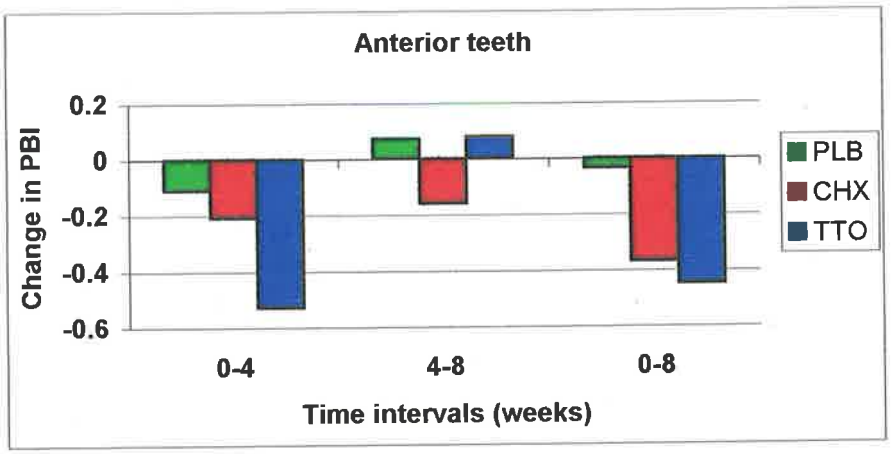
	PLB				CHX				TTO			
Time intervals (weeks)	n	mean	SD	P value	n	mean	SD	P value	n	mean	SD	P value
0 – 4	15	0.15	0.76	0.450	18	0.17	0.45	0.120	16	0.56	0.51	**0.001**
4 – 8	15	-0.11	0.75	0.582	18	0.06	0.72	0.702	16	0.02	0.70	0.874
0 – 8	15	0.04	0.75	0.828	18	0.24	0.69	0.162	16	0.58	0.56	**0.001**

Table 4.29 Descriptive statistics for PBI, anterior teeth.

	PLB				CHX				TTO			
Time intervals (weeks)	n	mean	SD	P value	n	mean	SD	P value	n	mean	SD	P value
0 – 4	13	0.11	0.92	0.649	15	0.21	0.69	0.212	14	0.53	0.53	**0.001**
4 – 8	13	-0.07	0.92	0.757	15	0.16	0.79	0.410	14	-0.08	0.77	0.676
0 – 8	13	0.03	0.74	0.859	15	0.37	0.77	0.058	14	0.45	0.72	**0.024**

Figure 4.2 a & b Average changes in PBI over weeks 0-4, 4-8 and 0-8, in posterior and anterior teeth.





All groups showed a decrease in PBI for weeks 0-4 and 0-8 in both anterior and posterior teeth. Small increases were noted in the PLB group (posterior teeth) and PLB and TTO groups (anterior teeth) during weeks 4-8. The TTO group exhibited the largest decrease in both data sets between weeks 0-4 and 0-8, these changes were statistically significant. The PLB group consistently exhibited the smallest decreases in PBI over all time intervals.

T-tests for PBI using data from anterior and posterior teeth with shallow and deep pockets

T-tests were carried out using data sorted according to pocket depth. Teeth with initial pocket depth < 5 mm were grouped into the "shallow" data set and teeth with initial pocket depth ≥ 5 mm were grouped into the "deep" data set. These data sets were separated further into data sets consisting of information from anterior and posterior teeth with shallow and deep pockets. When ANOVA and t-tests were carried out, the results for GI, PSS and PBI were very similar to the previous section (data from anterior and posterior teeth). This was probably because some teeth had both deep and shallow pockets, and were consequently included in both data sets.

T-tests for PBI using data from lingual and buccal sites

Table 4.30 Descriptive statistics for PBI, lingual sites.

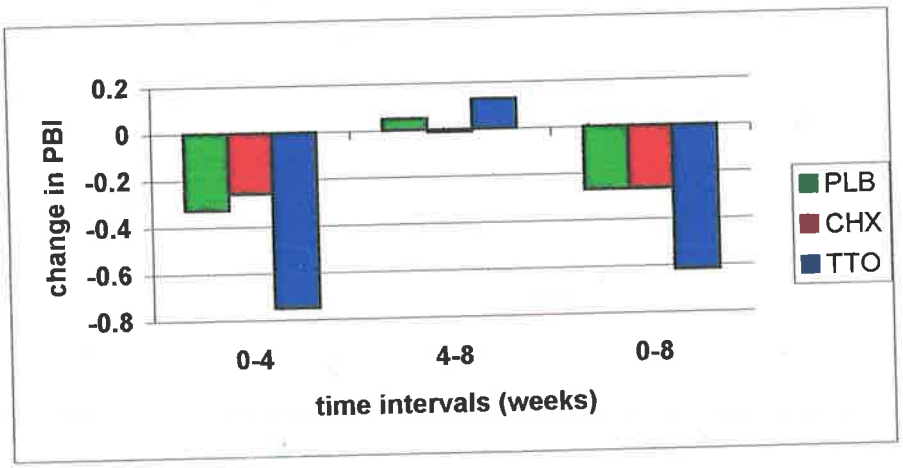
	PLB				CHX				TTO			
Time intervals (weeks)	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**
0 – 4	15	0.26	0.87	0.27	15	0.06	0.28	0.658	15	0.36	0.74	^0.069
4 – 8	15	-0.18	0.87	0.433	15	0.04	0.67	0.786	15	-0.24	0.68	0.172
0 – 8	15	0.07	1.11	0.794	15	0.11	0.71	0.538	15	0.12	0.74	0.528

No significant changes were seen in any group, for any time period. The TTO group's changes in weeks 0-4 approached significance. The TTO group had the largest changes (decrease) in the 0-4 and 0-8 time periods.

Table 4.31 Descriptive statistics for PBI, buccal sites.

	PLB				CHX				TTO			
Time intervals (weeks)	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**
0 – 4	15	0.33	0.69	^0.089	18	0.26	0.33	**0.037**	16	0.75	0.46	**0.000**
4 – 8	15	-0.05	0.87	0.806	18	0.01	0.05	0.900	16	-0.13	0.41	0.226
0 – 8	15	0.27	0.81	0.212	18	0.27	0.27	0.126	16	0.62	0.56	**0.001**

Figure 4.3 Average change in PBI over weeks 0-4, 4-8 and 0-8, buccal surfaces



At buccal sites, the TTO group had the most significant and largest changes in PBI (2) (0.75 and 0.62 for weeks 0-4 and 0-8 respectively). It also exhibited the largest change

(increase) for weeks 4-8, however this was small in magnitude. The PLB and CHX groups exhibited similar changes over the 8 weeks.

Summary of t-tests for PBI

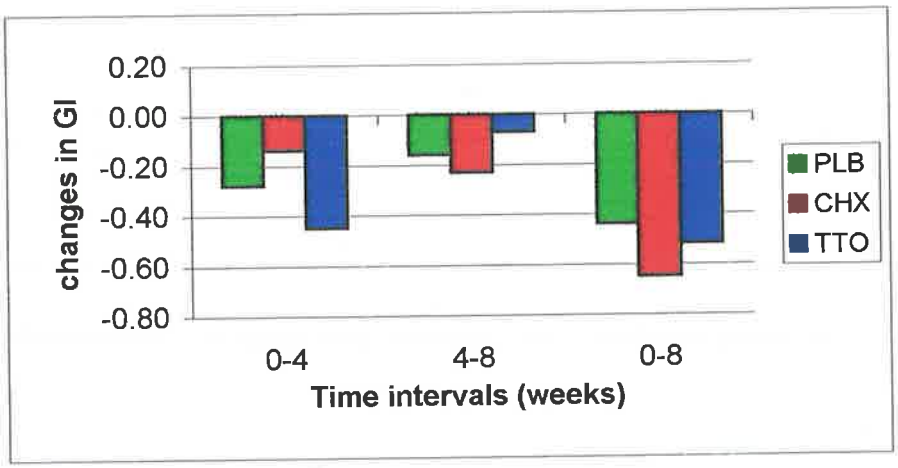
The TTO displayed the greatest change in PBI for the all data sets during weeks 0-4 and 0-8. This occurred in 10 time periods out of 15 analysed (3 time periods for 5 data sets). Except for lingual surfaces, all of these changes were statistically significant (8). The CHX group had 5 time periods with the greatest change, of these none were statistically significant. These all occurred in the 4-8 week period and were small in magnitude. The PLB group had the least (0).

T-tests for GI

Table 4.32 Descriptive statistics for GI, all teeth.

	PLB				CHX				TTO			
Time intervals (weeks)	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**
0 – 4	15	0.28	0.56	0.074	18	0.14	0.44	**0.001**	16	0.45	0.53	**0.004**
4 – 8	15	0.16	0.30	0.058	18	0.23	0.34	**0.010**	16	0.07	0.40	0.466
0 – 8	15	0.44	0.57	**0.009**	18	0.65	0.43	**0.000**	16	0.52	0.50	**0.001**

Figure 4.4 Average changes in GI, all teeth.



All groups had decreases in GI. In week 0-4 the TTO group showed the largest decrease but the CHX group had the greatest decrease between weeks 0-8. The CHX group had greatest number of significant changes in GI (3). The PLB group had the lowest number of significant changes (1) and the smallest change (0.44) in GI.

T-tests for GI using data from posterior and anterior teeth

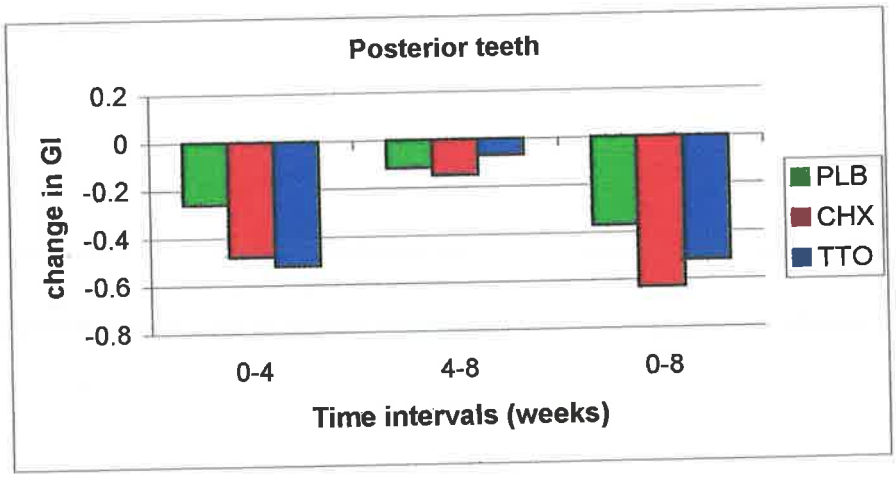
Table 4.33 Descriptive statistics for GI, posterior teeth.

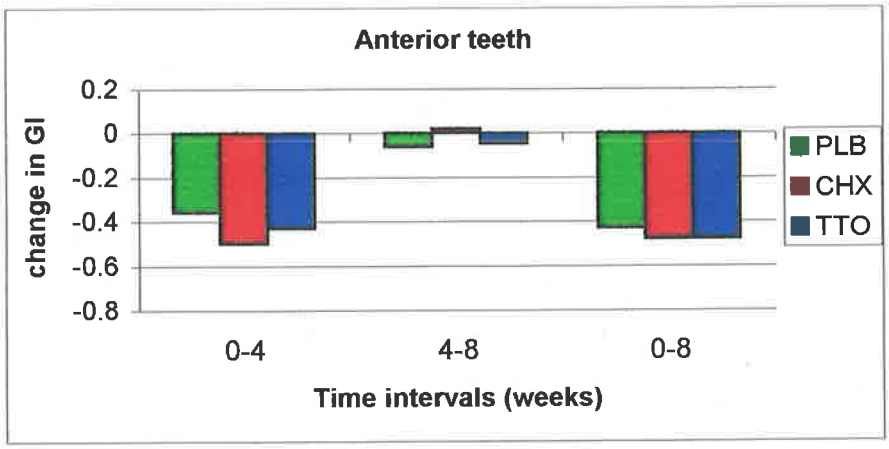
	PLB				CHX				TTO			
Time intervals (weeks)	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**
0 – 4	15	0.26	0.74	0.201	18	0.48	0.60	**0.004**	16	0.52	0.49	**0.001**
4 – 8	15	0.12	0.54	0.414	18	0.15	0.46	0.174	16	0.07	0.37	0.940
0 – 8	15	0.37	0.65	**0.043**	18	0.63	0.57	**0.000**	16	0.52	0.51	**0.001**

Table 4.34 Descriptive statistics for GI, anterior teeth

	PLB				CHX				TTO			
Time intervals (weeks)	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**
0 – 4	13	0.36	0.85	0.150	15	0.50	0.57	**0.005**	14	0.43	0.65	**0.029**
4 – 8	13	0.06	0.71	0.754	15	-0.02	0.50	0.877	14	0.05	0.50	0.072
0 – 8	13	0.43	0.62	**0.028**	15	0.48	0.63	**0.010**	14	0.48	0.71	**0.025**

Figure 4.5 a & b Average change in GI, posterior and anterior teeth





Most groups showed a decrease in GI in both anterior and posterior teeth (except the CHX groups in weeks 4-8, which exhibited a small increase in anterior teeth). The CHX and TTO groups showed larger decreases in GI compared to the PLB groups, more so in the posterior than anterior teeth. The TTO and PLB groups exhibited the greatest number of significant changes in posterior teeth, whereas the TTO and CHX groups exhibited the greatest number of significant changes in anterior teeth.

T-tests for GI using data from lingual and buccal sites

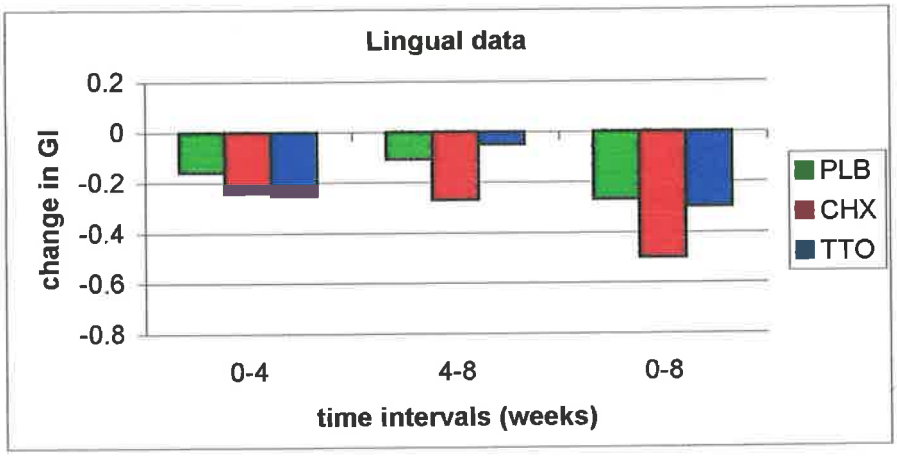
Table 4.36 Descriptive statistics for GI, lingual sites

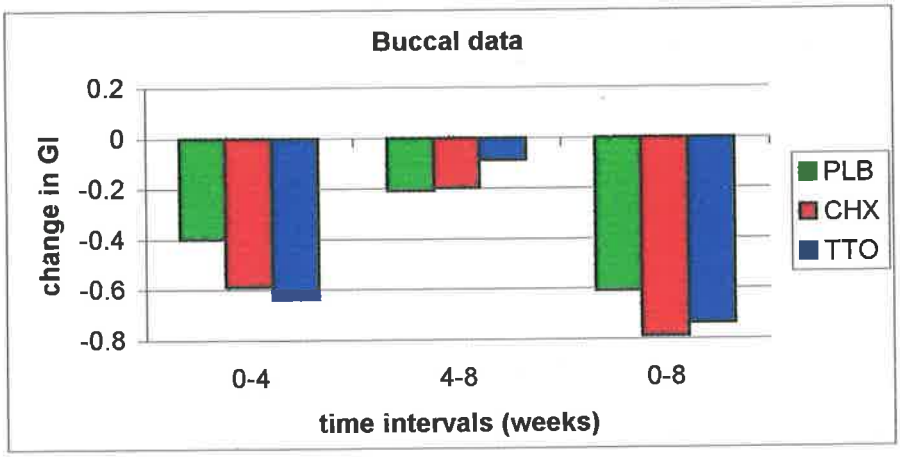
	PLB				CHX				TTO			
Time intervals (weeks)	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**
0 – 4	15	0.16	0.62	0.344	18	0.24	0.43	**0.031**	16	0.25	0.60	0.117
4 – 8	15	0.11	0.35	0.234	18	0.27	0.37	**0.008**	16	0.05	0.44	0.627
0 – 8	15	0.27	0.61	0.105	18	0.50	0.52	**0.001**	16	0.30	0.59	^0.057

Table 4.36 Descriptive statistics for GI, buccal sites

	PLB				CHX				TTO			
Time intervals (weeks)	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**
0 – 4	15	0.40	0.65	**0.030**	18	0.59	0.40	**0.001**	16	0.64	0.53	**0.000**
4 – 8	15	0.21	0.38	**0.049**	18	0.20	0.21	^0.053	16	0.09	0.45	0.410
0 – 8	15	0.61	0.68	**0.004**	18	0.79	0.61	**0.000**	16	0.74	0.55	**0.000**

Figure 4.6 Average changes in GI for lingual and buccal data





All groups exhibited decreases in GI on lingual and buccal surfaces. Smaller changes were seen on lingual than buccal surfaces. The CHX group had the largest decreases in both surfaces. In lingual sites, the CHX group had more significant (3) and larger (0.50) changes in GI than the PLB and TTO groups. The TTO group's change in GI after 8 weeks (0.30) approached statistical significance. At buccal sites, the PLB group had the most significant changes (3) compared to the TTO and CHX groups (2). However the TTO and CHX groups had the larger changes in GI (0.74 and 0.79 respectively) than the PLB group (0.61) over 8 weeks.

Summary of t-tests for GI

Overall the CHX group had the greatest change in GI in many data sets (8 time periods). Of these, 7 were statistically significant. The TTO group was next with 6 time period with the greatest change. Of these 5 were statistically significant. The PLB group had the least (2), of these one was statistically significant.

T-tests for PSS

Table 4.37 Descriptive statistics for PSS, all teeth

	PLB				CHX				TTO			
Time intervals (weeks)	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**	**n**	**mean**	**SD**	**P value**
0 – 4	15	0.16	0.63	0.348	18	0.09	0.69	0.550	16	-0.13	0.58	0.392
4 – 8	15	0.07	0.92	0.757	18	0.10	0.59	0.501	16	0.07	0.35	0.939
0 – 8	15	0.23	0.97	0.369	18	0.19	0.87	0.360	16	-0.12	0.72	0.507

There were no significant changes in PSS in any group for any time period. The changes seen in all groups were small. The PLB group exhibited the largest changes over 8 weeks. The TTO group was the only group to have increased plaque scores for time weeks 0-4 and 0-8. The remaining data sets also showed no statistically significant changes in any group.

Overall the CHX group had the greatest change in PSS in many data sets (7), the PLB group was next (6), and the TTO group had the least (1). However none of these were statistically significant.

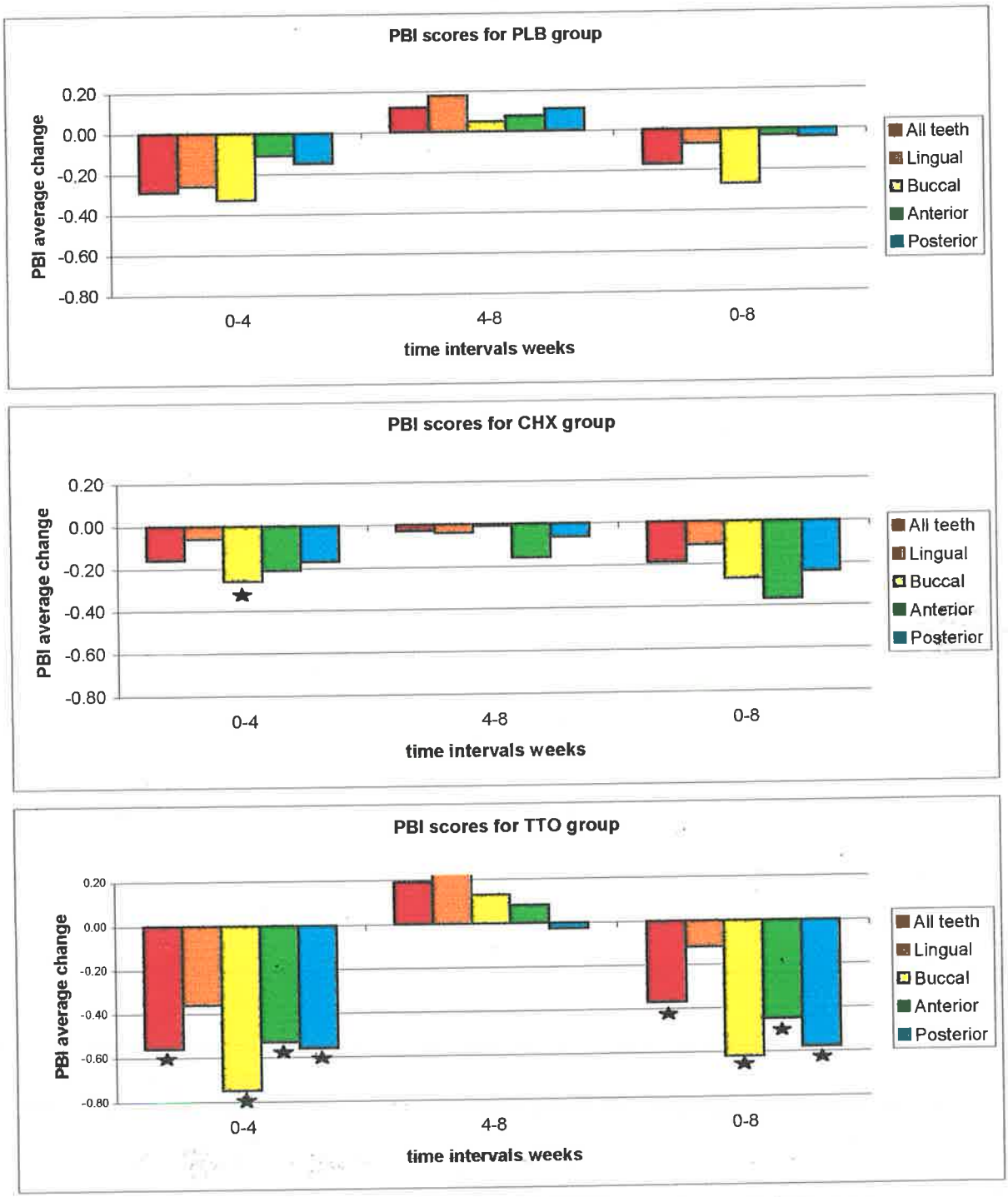
T-tests for PD/PAL

Although some statistically significant changes did occur, no significant trends were noted in these data sets. Changes in PD and PAL ranged between 0 mm – 0.8 mm which is below the minimum measuring unit on the probe used in the study.

Collation of results from t-tests by group and location

The following figures summarise all t-tests, giving an indication of the differences between the data sets (★ denotes statistical significance).

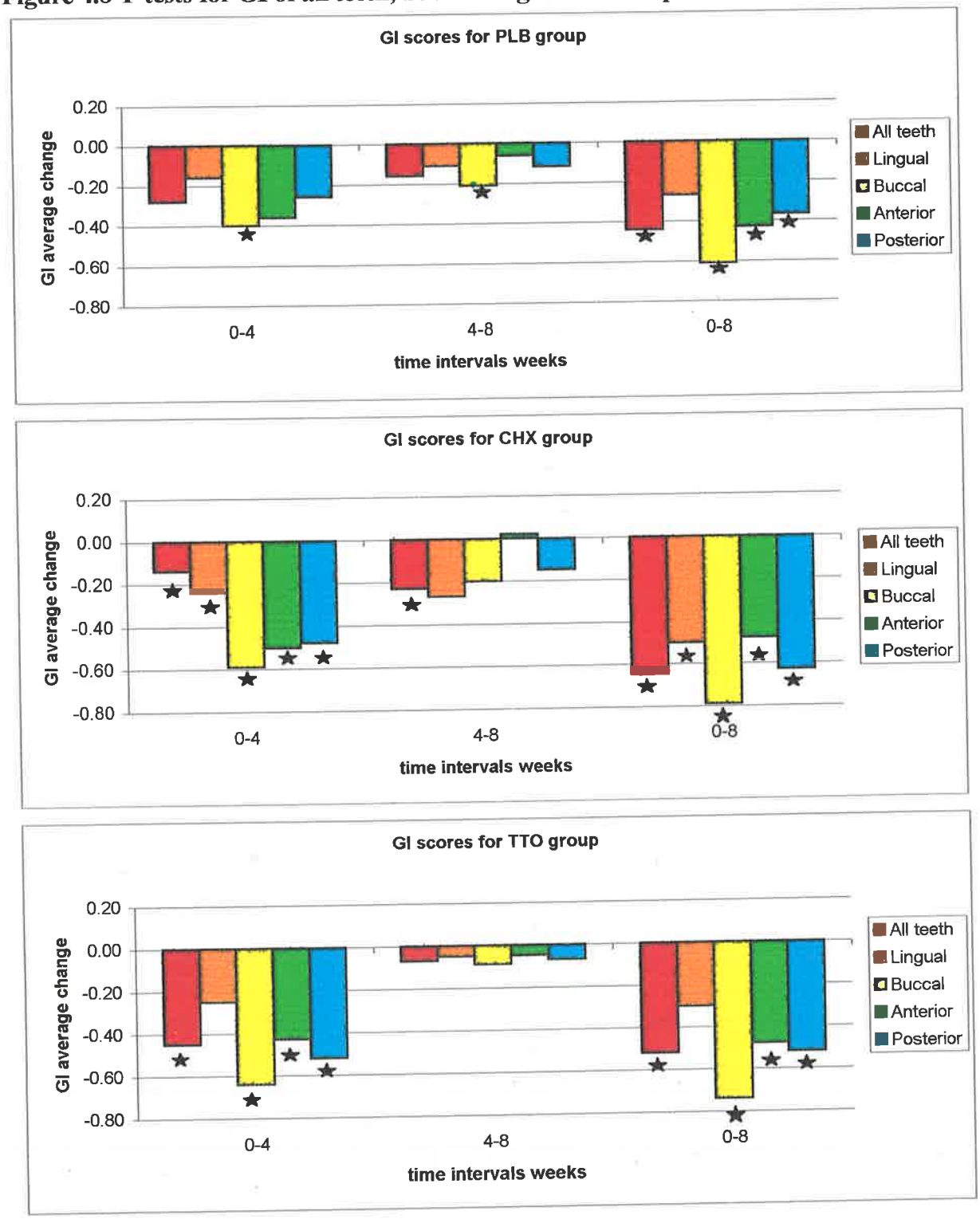
Figure 4.7 T-tests for PBI of all teeth, buccal /lingual & ant/posterior data sets.



Small changes in PBI were noted for the PLB and CHX groups. The TTO group exhibited the largest changes. In all groups the largest changes in PBI occurred in the

buccal surfaces (apart from CHX weeks 0-8). Anterior and posterior teeth showed a similar magnitude of change in PBI.

Figure 4.8 T-tests for GI of all teeth, buccal /lingual and ant/posterior data sets.



The smallest changes were seen in the PLB group, the TTO and CHX groups showed the largest. The buccal aspects showed the greatest change. Similar to the PBI data set, the anterior and posterior teeth showed a similar magnitude of change in GI. The changes for PSS, PD and PAL were very small and no discernable pattern was noted

Taste rating

Table 4.38 Descriptive statistics for subjects' evaluation of taste of gels.

Week	Group	Mean	SD	n	Lower bound (95% CI)	Upper bound (95% CI)	P value (ANOVA)
week 4	PLB	2.73	0.96	15	2.20	3.27	0.158
	CHX	2.67	0.97	18	2.18	3.15	
	TTO	3.25	0.86	16	2.79	3.71	
week 8	PLB	2.87	0.92	15	2.36	3.37	0.743
	CHX	2.94	0.87	18	2.51	3.38	
	TTO	3.13	1.09	16	2.55	3.70	

The TTO group had the highest taste score (lower scores ie 1-2 represented poor to average taste, 3-4 represented good to excellent taste) compared to the other groups for both time periods. The differences between the groups were greater after the first 4 weeks than after the 8 weeks.

These results reflect reports from subjects, where many reported that they became accustomed to the taste of the gel they were using.

Photographic results

During the course of the study after processing photographs of several subjects after week 0 and week 4 discrepancies in the colour, brightness and contrast of the photographs was noted. Although the problem was eventually resolved the variation

between time periods were so great that objective comparison was not possible and therefore the information gathered from this medium was left out of the study.

Null hypotheses

In consideration of the results, the following comments can be made about the study's null hypotheses.

- H_o 1: There is no difference between the 0.2% CHX gel and the 2.5% TTO gel in their anti-plaque effect. Although this hypothesis can be accepted since no statistically significant changes in PSS occurred in any group, the result may have been affected by poor patient compliance, oral hygiene and the presence of gross plaque and calculus from the beginning of the study.
- H_o 2: There is no difference between the 0.2% chlorhexidine gel and the 2.5% TTO gel in their anti-gingivitis effect. This hypothesis can be refuted if considering PBI, since TTO showed larger changes than the CHX group, however similar results occurred in GI for both groups.
- H_o 3: There is no difference between the placebo gel and the 2.5% TTO gel in their anti-plaque effect. This hypothesis can be accepted, although no statistically significant changes in PSS occurred in any group, indicating that further investigation in this area is required.
- H_o 4: There is no difference between the placebo gel and the 2.5% TTO gel in their anti-gingivitis effect. This hypothesis can be refuted since the TTO group showed larger improvements in PBI and GI compared to the placebo group.

Chapter 5

Discussion

This study investigated the effects of topical application of a TTO-containing gel on severe gingival inflammation in 49 subjects. While few changes occurred in most clinical parameters during the trial, significant changes were apparent in PBI and GI for the CHX and TTO groups. Within the limitations of the study, it was evident that the TTO-containing gel decreased the level of gingival inflammation more so than the positive and negative controls. Of particular interest, the improvements in PBI in the TTO group were not related to reduced plaque scores, perhaps indicating that the gel had a topical anti-inflammatory effect that was independent of the degree of plaque accumulation. Also, initial pocket depth did not influence the extent of improvement in PBI ie deep and shallow sites had similar changes.

PBI

It is difficult to relate index changes to clinical relevance. Unfortunately there are no "change thresholds" in the periodontal literature for indices that could be used as a guide when determining the clinical effectiveness of topically applied agents. A possible approach is to consider PBI changes of more than 1 unit, which are clinically measurable. Although the TTO group did not exhibit mean changes higher than 1 unit in any data set, however it did approach it for several data sets. For example, it reached a mean value of 0.751 for buccal surfaces (average change weeks 0-4), three times that achieved by the CHX group (0.256) and over twice that of the PLB group (0.328). The 95% confidence intervals for buccal surfaces showed a 1 unit change (range 0.492 – 1.009), indicating that clinically relevant/measurable changes occurred at buccal sites in the TTO group.

Instead of using mean changes to compare groups, an alternative approach to increase the clinical relevance of the statistical analysis would be to determine the number of sites with a change of one unit or more. This was carried out using data for PBI, GI, and PSS. Improvements were classified as a positive change in each index i.e. a change from the original score to a lower score. For PBI, the TTO group showed the largest number of improved sites for the following data sets: all teeth, posterior teeth, anterior teeth and buccal surfaces. All of these data sets (excluding the anterior teeth) had statistically significant improvements for the 0-4 week time period. The buccal surfaces showed the largest improvements in PBI. GI and PSS did not show any statistically significant improvements. However for GI, both the CHX and TTO groups exhibited the largest number of improved sites (more improvements than other groups) for weeks 0-4 and 0-8. The changes observed in PBI may indicate that TTO reduced existing gingivitis in subjects to a greater extent than occurred in the other groups. Comparison of these data with other researchers' work is not possible due to the lack of literature on the oral effects of TTO.

There were different responses in PBI in different data sets; a greater number of significant changes occurred in relation to the posterior teeth compared to anterior teeth. This was a somewhat surprising finding as it would be expected that more gel would be distributed to anterior teeth, as these are easier to access by brushing. Although posterior teeth showed more significant changes in PBI than did anterior teeth the magnitude of changes were similar (see Figures 4.2 a & b, page 79, 4.7, page 87). This difference was enough to make changes in posterior teeth statistically significant. The similarity of changes in anterior and posterior teeth may have been related to the way the teeth were allocated into groups as the premolars were allocated to the posterior teeth

group. As access to premolars by toothbrushing may be similar to anterior teeth (or at least better than molar teeth), improvements in the posterior teeth data set may have been predominantly due to changes related to the premolars. Buccal surfaces showed significant changes whereas the lingual surfaces did not. Varying responses in relation to different tooth surfaces have also been reported in CHX gel studies (Lennon & Davies 1975; Lie & Enersen 1986) with buccal surfaces showing more improvement in clinical parameters than the lingual or interproximal surfaces.

GI

At baseline, the groups did not vary significantly with respect to GI. Throughout the trial, no significant differences between group means or mean changes were detected in the ANOVA tests, indicating that the responses were similar or small, depending on the data set. Paired t-tests showed that the CHX group had the most time periods with the greatest improvement in GI (8); of which 7 were statistically significant, the TTO group was next (6) of which 5 were statistically significant and the PLB group had the least (2) of which 1 was statistically significant. This response in GI may indicate that CHX and TTO had similar effects in reducing gingival inflammation.

These results are similar to most CHX gel studies (Hoyos *et al.* 1977; Joyston-Bechal *et al.* 1984; Lie & Enersen 1986) but the magnitude of changes reported by these authors was greater than observed in the current study. A likely explanation is that, in the studies referred to, subjects had a scale and clean at baseline. Therefore, the changes seen were due to mechanical treatment in addition to the effect of CHX gel (see page 111 for further discussion of this factor). The finding in this study that the TTO group and the CHX groups had decreased PBI and GI scores may point to a clinically relevant

therapeutic effect because oral hygiene practices were poor and subjects had considerable deposits of supragingival/subgingival plaque and calculus throughout the study.

Comparison between the PBI and GI

There is no periodontal 'gold standard' index for assessing the degree of gingivitis or the treatment effects of various agents or treatment methods. Since GI has been used widely in studies investigating oral hygiene products and their effects on gingivitis, it was included in this study to permit some comparison between studies. However, there are significant limitations inherent in the GI which are neither acknowledged nor discussed in the periodontal literature. GI has fewer scoring criteria than PBI; it therefore provides less information and is less sensitive than the PBI to smaller changes in gingival inflammation (Table 5.1). The GI was the first index to score gingivitis and was formulated when the science of Periodontology was in its infancy. At the time, it was thought that colour changes in the gingival tissues preceded the development of BOP, whereas subsequent research has shown the reverse to be the case (Meitner *et al.* 1979; Hirsch *et al.* 1981). Gingival colour change is not necessarily an accurate indicator of gingival inflammation, as incipient gingival inflammatory lesions have BOP but do not necessarily have altered colour.

Table 5.1 Comparison of the GI and PBI

Gingival Index (Löe 1967)		Papillary Bleeding Index (Fischman 1988)	
0	normal gingiva	**0**	no bleeding
1	mild inflammation, slight change in colour, slight oedema, no bleeding on probing	**1**	only one bleeding point present
2	moderate inflammation, redness, oedema, glazing, bleeding on probing	**2**	several isolated bleeding points or a small area of blood
3	severe inflammation, marked redness and oedema, ulceration, tendency to spontaneously bleed	**3**	interdental triangle filled with blood
		4	profuse bleeding spreading toward the marginal gingiva

Other differences include the way in which gingivitis is described. The criterion for GI score 3 is very similar to the clinical signs and symptoms of ANUG (Horning & Cohen 1995) i.e. spontaneous bleeding, ulceration. Consequently GI scores of 3 should not be scored frequently in clinical trials assessing chronic gingivitis, rendering the GI with two criteria (0,1,2) to describe gingival inflammation and decreasing the index's sensitivity to measure changes in gingival inflammation. In this study, GI scores of 3 were recorded approximately as frequently as were scores 1 and 2 (data not shown), even though no subjects had overt signs of ANUG. The relatively frequent use of GI score 3 showed how the index was being interpreted during the study by assigning score 3 to the most severe cases of gingivitis rather than conforming to the letter of the index's criteria. This is probably a common occurrence in studies using GI.

The PBI is invasive in type, whereas GI has both invasive and visual components. Invasive indices assess the degree inflammation of gingival tissues using a probe to elicit bleeding. Visual indices use subjective visual assessments. Both types of indices have been shown to correlate with the histologic signs of inflammation (Greenstein *et al.* 1981). Engelberger *et al.* (1983) demonstrated the direct relationship between PBI

and the absolute amount of inflammatory infiltrate in gingival papillae. In addition, as PBI scores increased there was a definitive increase in the intensity of the infiltration. (Greenstein *et al.* 1981) carried out morphometric analyses of healthy and inflamed gingival tissues. Gingival sites were evaluated visually and for bleeding after probing using a controlled insertion pressure of 25 g (similar to that used in the current study). However in areas inaccessible for visual evaluation of inflammation, bleeding determinations provided a more objective diagnostic method for detecting the presence of inflammatory lesions. Probing the gingival tissues allows the assessment of the inflammatory state of the deeper gingival connective tissue whereas visual observations of gingivitis are limited to inflammatory changes that have become visible through the gingival tissues. Barnett (1996) stated that "the rationale for non-invasive GI's comes from the feeling that invasive indices are not suitable for longitudinal clinical trials". However the opposite may hold true for visual indices used in longitudinal studies. Maintaining the reproducibility of visual assessments i.e. degree of redness, oedema and glazing, between two time periods is difficult. Conditions used while assessing visual signs of gingival inflammation, such as the time of day and light sources and operator tiredness can affect the appraisal of colour by the operator.

Another confounding factor of indices with visual components is the inclusion of inflammation due to causes other than a bacterial aetiology i.e trauma from toothbrushing and flossing (Caton *et al.* 1988). One factor that has not been previously mentioned in the literature is the mis-identification of mucocutaneous lesions or other non-plaque associated gingivitis (viral, systemic factors) as plaque-induced gingivitis and their incorporation into the clinical assessments. This ultimately increases the potential of more false positive recordings by visual indices.

The changes seen in PBI in the present study could indicate that bleeding was a more sensitive measure of inflammation than colour change, particularly around the posterior teeth where direct visualisation of gingival tissues was difficult. Some authors disagree with this line of thought. Barnett (1996) suggested that although the inclusion of bleeding is commonly thought to confer objectivity to a gingival index, it can in fact be imprecise, subject to the interpretation of individual investigators. These inaccuracies can be due to:

- the amount of pressure applied to the tissues, greater than 0.25 N force causes an increase of false positives scores. This was not an issue in the current study since a constant force probe was used.
- the angle and depth of probe placement. In the current study probing was in a vertical direction to the base of existing pockets.
- the way in which the bleeding is elicited i.e. the probe may be held vertically or swept across the gingival margin. In the current study the probe was held vertically (in alignment with the long axis of the tooth) and inserted into the pocket without a sweeping motion.

Objections to invasive indices have also come from Lobene *et al.* (1986) and Ciancio *et al.* (1992) due to:

- disturbance of plaque and trauma to tissues affecting both plaque and gingivitis scores. The disturbance of plaque was possible in the current study, however, due to the use of a constant force probe and probing sites once only meant that trauma to gingival tissues was minimal. The assessment of clinical parameters 4 weeks apart meant that even if plaque was disturbed it was unlikely to have influenced plaque quantity at the following data collection time.

- obscuring of specific bleeding sites by blood flowing from probed site onto the opposite or adjacent tooth simultaneously. This was a problem experienced at times during the current trial.

Trauma from eliciting BOP has been considered a source of error when assessing successive bleeding scores (Feldman *et al.* 1982; Badersten *et al.* 1990). This occurred when replicate bleeding measurements were made 90 minutes apart. It was concluded that the reproducibility of successive bleeding scores was weak. This was not the case in the current trial where intra-examiner reproducibility (measurement made one day apart) was approximately 78% for GI (see results) and 79% for PBI regarding replication of scores (100% accuracy) and 97 and 93% with 1 unit of error respectively. In the current study, sites were probed once and a constant force probe was used to minimise trauma to the gingival tissues. The probing to determine PD and PAL was used as the stimulus to elicit bleeding to minimise trauma to gingival tissues. The sequence of assessment was PD/PAL, GI then PBI of a quadrant. This minimised the time between PD/PAL and GI/PBI assessments, decreasing the chance of blood from one site flowing over to another and thus affecting the score. However, some overflow of blood occurred infrequently, making the visual assessments of GI difficult due to excessive bleeding.

It is difficult to determine which index (PBI or GI) was more accurate in the current study. The differences between the indices that were apparent in the data were either due to measurement error or related to the accuracy of the index since the reproducibility of PBI and GI scores were approximately 70% (for absolute agreement of scores) and 90% within one 1 unit. The operator found that the PBI was a more objective index than GI. Standardisation of the assessment of degrees of redness over

long periods that is required in the GI was difficult. If the current study was extended, the inclusion of a clinical measure of gingival inflammation which increased objectivity would be warranted. This may include an assessment of subgingival pocket temperatures (Wolff *et al.* 1997) or cytokine measurements. Assessment of multiple cytokines ie IL-1, IL-8 and IL-6 in gingival crevicular fluid may provide more accurate information than just one cytokine since large cytokine networks exist which can influence each other's expression and are pleotropic at various concentrations (McGee *et al.* 1998).

PD and PAL

The PD and PAL of the three groups varied significantly at baseline. Scheffé tests showed that the TTO group had greater PD and PAL (approximately 1 mm for PD and PAL) than the CHX and PLB groups. This difference was maintained throughout the trial period. Few changes occurred in these parameters over the 8 week period. The variation seen in these parameters are likely to have been due to measurement error alone rather than a true clinical effect, since the assessment of PD and PAL with manual probes in untreated subjects, particularly in deep sites, has been associated with large measurement error and decreased ability to replicate measurements (Greenstein 1997).

There are inherent problems associated with longitudinal measurements of PD and PAL. A number of factors influence the degree of penetration of the probe into the pocket (Listgarten 1980; van der Velden 1980; van der Velden & de Vries 1980). Variations in reassessment of PD and PAL may be due to malposition of the probe due to anatomical features such as the contour of the tooth surface, variation in pressure applied on the instrument during probing and the degree of inflammation. Gingival inflammation allows the probe to penetrate the pocket epithelium and enter the underlying connective

tissue. Magnusson & Listgarten (1980) showed that in pockets deeper than 4 mm, a significant difference in histologic probe penetration was found between the treated and untreated sites. In non-treated sites, metal strips used for probing penetrated an average of 0.29 mm into the connective tissue apical to the junctional epithelium. An improvement in the PD and PAL parameters in any of the current treatment groups does not indicate reattachment/regeneration of periodontal tissues to the root, rather a decrease in the ability of the probe to penetrate the pocket epithelium and connective tissue. Although there were decreases in PBI/GI of the TTO and CHX groups indicating a decrease in inflammation, a corresponding decrease in PD/PAL did not occur. This may have been due to the fact that the changes in gingival inflammation were small and that manual probing was not sensitive enough to detect such changes.

Espeland *et al.* (1991) showed that when pocket depths were 3, 4-6 and 7 mm, the measurement errors in longitudinal assessments were 0.5, 0.7 and 1.3 mm respectively. In the current study, the changes in PD and PAL ranged from 0.2 – 0.4 mm in shallow sites (< 5 mm) and 0.4 – 0.8 mm (in deep sites i.e. PD ≥ 5 mm). The pressure sensitive periodontal probe used in the study had a diameter of 0.55 mm with 20 g (0.20 N) required to displace the mobile component. Keagle *et al.* (1989) found that a probe with a diameter of 0.60 mm was able to most consistently discriminate variation of gingival health status, and Bulthuis *et al.* (1998) concluded that, for the optimal assessment of the attachment level in inflamed periodontal conditions, a tapered probe with a tip diameter of 0.5 mm which exerted a controlled probing force of 0.25 N may be the most suitable. The probe used in the current study falls within these parameters. Ideally, an electronic probe such as the Florida Pocket Probe®, Florida Disk Probe®, or Peri Probe®

should have been used, since these have been shown to have better reproducibility and accuracy than conventional probes (Samuel *et al.* 1997).

The measurement of PAL was taken as the measure from the CEJ to the base of the periodontal pocket. In some cases when the CEJ was not visible at sites of overt hyperplasia or swelling, the adjacent CEJs were used as a guide to the position of the CEJ on the tooth being assessed. This may have been a source of error in consecutive measurements of PD and PAL. Measurement of PD and PAL were made difficult in some instances because subjects did not receive a scale and clean prior to commencement of the study.

PSS

Information gathered from the current trial and the limited literature indicates that TTO's antibacterial effects, although potent *in vitro,* do not translate to inhibition of plaque formation *in vivo*. Few changes occurred in the PSS parameter, including in the CHX group; no changes in PSS reached statistical significance. When considering intragroup changes, the CHX group had the most time periods with the greatest change in PSS (7), the PLB group was next (6), and the TTO group had the least (1). The TTO group showed some increases in PSS (compared with the CHX and PLB groups which had small decreases) in the different data sets. However, these changes were small and were well below the incremental units used to assess PSS.

There are only two published studies assessing TTO's effects on oral bacteria and on plaque formation *in vivo*. The response in PSS of the TTO group in the current trial was similar to other studies that have investigated TTO as an oral antibacterial agent (Rogers & Gully 1999; Arweiler *et al.* 2000). However, direct comparison of the results is not

possible because the TTO vehicle in those investigations was a mouthwash rather than a gel and the studies' protocols were very different. Rogers & Gully (1999) showed that a TTO containing mouthwash was effective in decreasing salivary bacterial counts for approximately ½ hour. This decrease was comparable to the CHX control, but after 6 hours, salivary bacterial levels nearly returned to normal whereas the activity of CHX persisted. This may indicate the lack of substantivity of TTO components. Similar results were seen in a clinical trial of TTO mouthwash. (Arweiler *et al.* 2000) used a crossover study design with a washout period of 3 days in 8 subjects. In the first week, water was used as a mouthwash in the absence of oral hygiene for 4 days, CHX mouthwash was used in the second week and TTO mouthwash in the last week. Plaque index and plaque areas were scored, as was vital fluorescence to detect vital bacterial colonies. The TTO containing mouthwash had no effect on the quantity or quality of supragingival plaque.

The CHX group's plaque scores in this study did not change significantly. This is contrary to the findings of clinical studies which also used CHX gel, although, clinical effects of CHX gel on plaque have been variable depending on the frequency of use, concentration used and whether or not subjects were treated or untreated at the beginning of the study making comparison between studies difficult (Hansen *et al.* 1975; Lennon & Davies 1975; Hoyos *et al.* 1977; Joyston-Bechal *et al.* 1984; Lie & Enersen 1986). The majority of CHX gel studies show a decrease in plaque scores which were usually statistically significant. The changes in GI or bleeding indices were not as large as those for plaque scores but were usually significantly different from the placebo groups (Hansen *et al.* 1975; Joyston-Bechal *et al.* 1984; Lie & Enersen 1986).

Some of these studies showed that the majority of treatment effects occurred early in the observation periods of the studies (0-4 weeks) possibly indicating decreased compliance later in the studies or that the end point of treatment effects had been reached i.e. improvements in clinical parameters reached a plateau after which no further improvement was possible (Hoyos *et al.* 1977; Joyston-Bechal *et al.* 1984). This observation was reflected in the current study where the majority of changes in PBI and GI were seen in the 0-4 week time period.

In this study only quantitative changes in plaque were measured. It is possible that some qualitative changes occurred in the subjects' plaque. If the study was extended it may be possible to include microbiological analysis via culturing, DNA probes or vital fluorescence techniques to determine changes in the profile of the biofilm.

Reasons for limited PSS activity in the CHX group

Several factors may have contributed to the limited changes seen in PSS, particularly in relation to the CHX group. Renton-Harper *et al.* (1998) showed that the timing of brushing episodes affected plaque scores taken the next day and that there was a need to standardise the time between plaque scoring and the previous tooth brushing. Ideally, appointments for all subjects in the trial should have been at the same time of day, but this was not always possible because of clinic access and subject availability. This may have affected plaque growth rates to a small degree since times between brushing episodes may have varied due to unsupervised applications of test agents. However, this should not have made a great difference in the CHX group due to CHX's substantivity and the fact that mature plaque takes days rather than hours to develop. Also, if subjects were using their toothbrushes effectively, plaque should have been kept to a minimum, regardless of the gel used. (Lang *et al.* 1973) showed in a group of subjects with regular

oral hygiene measures formed every 12 hours, that PI and GI scores remained low (below 1.0 and 0.25 respectively).

In some studies, a few subjects have consistently high or low plaque scores, regardless of the oral hygiene products used. For example, in a study by Lennon and Davies 1975, one subject was shown to have high PI (plaque index) scores at baseline compared with other subjects and after use of either CHX gel or the placebo gel the PI score did not change significantly. Other subjects showed larger decreases in plaque when using CHX gel. This may have been due to a higher plaque formation rate in this individual (or non-compliance). The opposite holds true for subjects who had low levels of plaque at baseline and maintained low levels even when using the placebo. This observation could be due to a variation in compliance and toothbrushing efficiency or due to the inherent plaque formation rate (high or low plaque formation rates) in each individual. This phenomenon has been reported previously (Simonsson *et al.* 1987). The factor of high and low plaque formation rates should ideally be considered when selecting subjects for trials assessing oral chemotherapeutics that can potentially affect plaque formation. High and low plaque formers could be separated into groups or perhaps only high plaque formers are selected to test periodontal chemotherapeutics. When using small group sizes, this effect can be accentuated if the majority of subjects are either high or low plaque formers. Categorisation of subjects prior to commencement of the study may have been warranted, but increased time and cost would have complicated the study. In addition changes in periodontal parameters in subjects waiting to commence the trial can occur while the categorisation is being undertaken.

Another factor that possibly contributed to the lack of plaque score changes in the current trial may have been poor compliance with gel use. An indirect indicator of

compliance may have been the time taken by subjects to use the gel over 4 weeks. The amount of gel per tube had been calculated by weighing an amount of gel equivalent to the length of the issued toothbrush head and calculating the weight needed for twice daily use for 4 weeks. The use of gel varied greatly. The majority of subjects claimed that the amount of gel supplied was sufficient for 4 weeks, with little gel remaining. A few subjects reported that they had a lot of gel remaining after 4 weeks, possibly indicating poorer compliance. The opposite may have been true for subjects who used all the gel within 3 weeks. Subjects were informed at the first appointment that if their gel was used prior to their next appointment, they should contact the research centre to arrange for more gel to be forwarded to them. Most of the subjects followed this instruction but a few did not (3 subjects), leaving them with no gel for several days. Subjects were specifically asked not to use other oral hygiene products during the course of the study, but as this was an unsupervised trial, this could not be monitored. Some subjects might have used toothpaste in addition to the gels. This may have occurred with subjects who disliked the taste of the CHX gel and preferred toothpaste; some subjects may not have used CHX gel or used it in combination with toothpaste. In the CHX group, this may have caused inactivation of the CHX due to divalent bonding with fluoride and other components of toothpaste and resulted in a limited effect on plaque formation.

Another consideration was the fact that subjects had pre-existing plaque and supragingival/subgingival calculus deposits which made effective oral hygiene difficult and facilitated plaque growth. Using disclosing solution on teeth with significant calculus deposits would result in staining irrespective of plaque formation, confounding plaque measurements. This would have affected all groups' recordings.

Regardless of the agent used, if a subject's tooth brushing ability was adequate, minimal plaque should have been detected on most tooth surfaces. This should have resulted in decreased plaque scores, as seen in placebo groups in other CHX gel studies (Hansen *et al.* 1975; Lennon & Davies 1975; Hoyos *et al.* 1977; Joyston-Bechal *et al.* 1984; Lie & Enersen 1986). This effect may represent the Hawthorne effect. It was evident in this study that subjects carried on with their usual standard of oral hygiene, which was poor.

Taste of the gels

Subjects feedback about the taste of the gels was assessed at weeks 4 and 8. There were no statistically significant differences between the gels regarding taste. However, at week 4, the TTO group had a higher mean score than the PLB and CHX groups respectively. At week 8 this relationship changed, the TTO maintained the best score with CHX second and PLB the worst. The differences between the TTO and PLB and CHX groups at week 8 were smaller than week 4 indicating that, over time, subjects became accustomed to the taste of the gel supplied. This result is surprising considering the poor taste of CHX gel (Ciancio 1992). These results may indicate that TTO gel had a more favourable taste than the PLB and CHX gels, however the parameter used to assess favourable taste was very subjective. Regardless of this a crossover study design may be more accurate for taste perception.

Trial related issues

Industry related issues

This trial was funded by industry, which also provided the agents used. Two months after the study commenced, the company funding the research went into liquidation, and

funding was terminated. Therefore the number of subjects that could be recruited was significantly decreased. The number of subjects initially aimed for was 90, but due to financial restrictions, only 49 subjects completed the study. Nine subjects dropped out (58 originally recruited), these subjects either did not attend the first appointment (3 subjects), attended the first appointment only (5 subjects) or attended the first and second appointments only. A decreased n value meant that the power of the statistical analyses decreased. Power is the probability that a statistical test will reach a particular correct conclusion eg the conclusion that H_A is true when in fact it actually is (Knapp & Miller 1992). Power is therefore analogous to sensitivity of a diagnostic test. By decreasing the power of statistical tests type two errors increase ie more false negative results. This means that H_O is accepted instead of H_A when it is true. For this study, significant changes in clinical parameters may have been missed.

Issues associated with the statistical methodology

ANOVA tests were used to identify statistically significant differences between groups, Scheffé's tests identified group(s) whose means varied significantly from the other group(s) and paired t-tests were used to assess intra-group variations. These analyses used mean differences to identify treatment effects. The advantage of mean differences is that they allow easy assessment of treatment differences (Hujoel *et al.* 1993) and are therefore commonly used in most periodontal research. However, statistically significant mean changes do not necessarily reflect clinically relevant events, leading to interpretation difficulties. For example, some groups showed statistically significant changes in PD ranging from 0.20 to 0.25 mm. The probe used in the current study had graduations of 1 and 2 mm and could not directly measure these changes, making it

difficult to quantify the clinical relevance of statistically generated changes. It is therefore important to temper the statistical data with the reality of the clinical situation.

Confidence intervals (95%) were reported to give an indication of the range of changes in a group and increasing the information about data sets. Comparisons of treatment effects are possible not only in terms of mean differences but also in relation to highest and lowest changes (see page 91).

There are alternatives to using mean differences to assess clinical data. One is the use of relative risk which measures the risk for disease progression i.e. increase in PD, GI, PBI, in the control groups relative to the risk for site specific disease progression in the treatment group. Relative risk is a widely used measure of association in clinical epidemiology and can be readily interpreted by both clinicians and patients. It reflects how much more likely treatment A results in success than treatment B (Hujoel *et al.* 1993). An improvement to the statistical analysis of data in the current trial may have been the inclusion of relative risk measures.

Subject selection and allocation into groups

The subjects in this trial were randomly assigned to the three treatment groups without reference to their baseline data. However, the selection criteria for entry into the trial did permit some standardization of baseline scores i.e. only subjects with severe gingival inflammation (GI score of 2 - 3) and at least 4 teeth (minimum of 20 teeth) with 5 mm pockets in at least one site met the selection criteria. These criteria were in agreement with Chilton & Fleiss (1986) who suggested that, in therapeutic trials where the goal of the agent is to reduce existing levels of gingivitis, subjects should show at least minimal signs of disease or plaque accumulation. Chilton & Fleiss (1986) also

suggested that subjects should be stratified into low, medium or high levels of disease and then randomly distributed amongst the different treatment groups. The stratification of subjects was considered to increase the efficiency of the clinical trial and allowed for more information to be obtained on a test agent's effectiveness.

In the current study, stratification of subjects was not carried out because the process would have added an additional layer of complexity and cost to the study. Some subjects may have dropped out before the study proper commenced because of the time delay occuring while recruiting sufficient numbers into groups. Given the difficulty that was encountered in attracting suitable subjects in the recruiting process, it is estimated that baseline measures may have been completed for all subjects after 3-4 months. Changes in clinical parameters during this period before commencement of the trial could have occurred due to loss of teeth, dental treatment elsewhere, disease progression etc, rendering baseline data useless. As it was, subjects began the trial immediately after measurement of their baseline data.

Separation of data into data sets

Subjects' cleaning abilities are determined by tooth position and surface, manual dexterity and motivation. Sites with little plaque accumulation relate to surfaces/teeth which are easy to access or which are included in a subject's routine toothbrushing habit. A difference in the ability of subjects to perform oral hygiene measures would impact on the distribution of gels to different tooth surfaces. Lang *et al.* (1973) found a difference in the distribution of plaque deposits in a group of subjects brushing twice a day. Plaque was rarely detected on the labial surfaces on any tooth except for second molars. On the lingual/palatal surfaces, molars displayed plaque on a regular basis and

plaque was noted occasionally on mandibular canines and premolars. Similarly, during the current trial it was the examiner's perception that there were more changes in gingival parameters relating to anterior than posterior teeth (although the data analysis showed otherwise) and in buccal compared with lingual surfaces in some subjects. Comparable observations exist in the literature where treatment effect varied relative to different tooth surfaces. For example Hansen *et al.* (1975) who examined the effect of CHX-containing gel on oral cleanliness and gingival health in young adults found that the improvement in plaque scores was greater at buccal and lingual than interproximal surfaces and the majority of change in GI occurred in buccal sites rather than lingual or proximal sites. The differences were suggested to be due to variations to subjects' cleaning ability as buccal surfaces were more accessible to brushing than lingual surfaces (Saxen *et al.* 1976).

Data were separated by tooth, surface and pocket depth. This was done to determine if differences in clinical effects occurred in the clinical parameters (particularly PD and PAL) between shallow and deep sites, since pocket depth can determine degree of bleeding upon probing, infiltrated connective tissue and diminished effectiveness of oral hygiene (Greenstein 1997). Improvements may be easier to detect in deep rather than shallow sites, because larger changes in PD or PAL may occur in initially deep sites. This is because small changes are required to produce health in shallow sites after which no further change is possible. Initial pocket depth did not affect the extent of changes in PBI in the TTO group. This may indicate that TTO was penetrating the gingival tissues since entering these deep sites from the pocket orifice was unlikely.

Treatment vs no treatment prior to commencement of study

Protocols not carrying out scaling and cleaning at baseline are regularly used for trials of periodontal chemotherapeutics (Chilton & Fleiss 1986), although it is surprising that many studies carry out treatment at baseline. Treatment (scale and clean) of subjects prior to a study must itself influence the degree of gingival inflammation, so that improvement in periodontal parameters cannot be solely assigned to the effects of the test agent. In studies in which scaling and cleaning is carried out at baseline, the effect of a test agent on plaque formation does not translate to an alteration of established clinical plaque levels. In this environment, the agent has to attack the biofilm in its entirety rather than prevent plaque formation. There are few agents which are effective in improving the clinical situation even in the presence of plaque, calculus, gingivitis and periodontitis (the real world situation). Studies which incorporate treatment prior to baseline measurements in an attempt to standardize levels of plaque and gingivitis between subjects can only assess the test agent's ability to prevent plaque formation and/or disease onset and progression. In studies which combine treatment and the use of test agents at baseline, the results will reflect response of the periodontal tissues to treatment in addition to the effects of the test agent. However, there is little or no mention of the effect of the treatment factor on measured parameters in many studies. Investigations which attempt to assess the therapeutic qualities of an active agent, but which include the treatment of subjects prior to the commencement of the study must have difficulty attributing clinical results solely to the agent being tested.

Significance of clinical results where the mean improvement is less than the error rate

The error rate becomes important when trying to determine if statistically significant changes are true or can be attributed to the examiner's error. In the study 100%

accuracy rates for GI and PBI ranged between 80 and 79% (error rate of 20 and 21%). Any results which showed changes less than the error rate should be interpreted cautiously since it is likely they represent examiner error rather than true clinical changes. If taking an example of results from buccal surfaces and posterior teeth (≥ 5mm PD) (which were statistically significant for ANOVA and/or T-tests) the changes in PBI and GI ranged from approximately 5-40% decrease in clinical parameter means for the CHX group and 20-41% changes. Apart from the changes in PBI for the CHX groups in posterior teeth the remaining changes were close to or above the error rate (see table 5.2).

Table 5.2 Percentage changes in PBI and GI scores during weeks 0-4 and 0-8 for TTO and CHX.

Buccal				**post teeth =+ 5mm**			
Percentage changes		**PBI**	**GI**			**PBI**	**GI**
wk 0-4	**CHX**	10.04%	29.50%	**wk 0-4**	**CHX**	5.75%	19.69%
wk 0-8	**CHX**	10.84%	39.50%	**wk 0-8**	**CHX**	6.51%	35.75%
wk 0-4	**TTO**	26.29%	31.37%	**wk 0-4**	**TTO**	20.88%	26.47%
wk 0-8	**TTO**	21.80%	41.18%	**wk 0-8**	**TTO**	20.15%	31.86%

Possible periodontal applications of TTO gel

One goal of periodontal therapy is the minimization of gingival inflammation by antibacterial therapy in the form of mechanical debridement and antiseptic/antibiotic administration (irrigation/topically applied devices). At best, topically applied periodontal chemotherapeutics (irrigation, chips, gels and fibres) may transiently reduce gingival inflammation when used in the absence of periodontal treatment. The efficacy of active agents placed into periodontal pockets is limited because of technical problems associated with placing them into deep sites and providing a sufficient reservoir to account for clearance by GCF flow (which is relatively high at inflamed sites).

Assessment of the value of local irrigation is made difficult by the large number of agents tested and the varied protocols that have been used to test them (Addy & Renton-Harper 1996). Studies have been concerned mostly with the topical use of chlorhexidine, tetracycline, minocycline or metronidazole; most trials have shown some short-term improvement in one or more periodontally related parameters, but this has also been reported for saline irrigation (Addy & Renton-Harper 1996). Fibres and chips containing high concentrations of active agents for placement into periodontal pockets were developed in an attempt to overcome these limitations, but comparison of effectiveness between studies remains difficult. The advantages and disadvantages of these products is beyond the scope of this discussion and the reader is directed to the following articles (Greenstein 1995; Greenstein & Polson 1998; Killoy & Polson 1998).

An alternative to the use of these topically applied antibiotic/antiseptics are NSAIDs. With the advent of anti-inflammatory drugs, interest grew about the uses of these drugs in the prevention of periodontal disease initiation and progression. Clinical studies have shown that the inhibition of inflammatory mediators using anti-inflammatory drugs either topically or systemically could produce favourable clinical and radiographic results (Offenbacher *et al.* 1992; Drouganis & Hirsch 2001). The majority of reports suggested that both topical and systemic administration of NSAIDs could decrease GI, PD, PAL and have protective effects on periodontal bone levels as determined by sequential radiographs.

Since decreased gingival inflammation occurred in subjects who used TTO without a concomitant decrease in plaque scores, the mechanism of activity was most likely to have been anti-inflammatory rather than antibacterial in nature. TTO may therefore be a unique agent for topical application to the gingival tissues as its properties are very

different to the antiseptics and antibiotics currently in use. The anti-inflammatory action of TTO may have been recognised many years ago by herbalists or medicine men of indigenous cultures. Interestingly, the delivery system that the Australian aborigines used to administer TTO is by external application or inhalation. The only way for essential oils to affect organs such as the lungs or nasal mucous membranes is by penetration of the epithelium to the tissues and into the blood stream. If TTO is readily absorbed after topical application into the gingival tissues and has anti-inflammatory properties, it would be a unique anti-gingivitis agent. It is easily applied, cheap and patients in other settings have reported that it decreased swelling and bleeding and made their gums feel more comfortable. Because of this positive experience, many patients have requested more TTO gel once they had completed the first sample.

Some of the components of TTO are known to have lipophilic properties which facilitate its diffusion through epithelium (Williams & Barry 1991) and possibly the deeper tissues. Should future studies confirm this property for the gingival tissues, self administered TTO gel would be a cost effective and simple method to control gingivitis and hopefully, periodontitis.

TTO gel could be used in patients after periodontal therapy between maintenance visits, where scale and cleaning or deplaquing occurs. This would ensure that increases in gingival inflammation between appointments may be limited by use of the gel. It may also be of use during treatment, particularly in early treatment phases where the patient is commencing periodontal treatment and has severe gingival inflammation or for relief of symptoms associated with gingivitis (bleeding or swelling) by patients in the form of an over the counter product.

However, suppression of an inflammatory process such as gingivitis, which has been considered by some authors to be protective against secondary stages of periodontal disease (periodontitis) (Page 1986), may not be entirely favourable. The pathogenesis of periodontitis involves altered immune/inflammatory responses. These can include inadequate responses which allow persistence of periodontopathogens or alter healing which increases susceptibility to the development or progression of periodontitis. Examples of these alterations may occur in patients suffering from early onset periodontitis with neutrophil dysfunctions or in smokers with severe chronic periodontitis but who have low levels of overt gingivitis (Hanioka *et al.* 2000), humoral and cellular immune systems and depreciated inflammatory systems (Kinane & Chestnutt 2000).

The effectiveness of a TTO-containing gel in the treatment of gingivitis and periodontitis needs to be assessed in long-term studies using either surrogate or true endpoints related to health or tooth survival. Ideally, tooth survival is a better measure of disease progression than surrogate endpoints such as PD or PAL due to the possibility of false negatives i.e. not detecting disease progression, because of inherent measurement errors. However, such studies would take many years to complete.

Proposed avenues of further study

The current study warrants following up or extending. Suggestions for improvement of the study design would include:

- increasing the number of subjects in each group to increase the statistical power of the study.

- increasing the concentration of the TTO in the gel formulation. This may allow for a greater concentration to be absorbed into the gingival tissues. Assessing the incidence of toxicity and allergic responses would be required since no guidelines on the maximum concentration for oral use is available.
- addition of an agent that increases the substantivity of the TTO to tooth surfaces, such as "Gantrez® " a copolymer used to increase the substantivity of triclosan.
- studying the periodontal effects of systemic administration of 1,8-cineole in capsule form "Soledum". Other components of TTO, such as terpinen-4-ol, may be considered as an active agent for systemic administration via capsules/tablets to influence periodontal attachment loss.
- investigating combinations of essential oils eg. TTO, thymol or peppermint oil
- alternatively, single components of the TTO gel such as terpinen-4-ol or 1,8-cineole may be isolated and tested individually for oral effects.
- investigating essential oils or their components as penetration enhancers (limonene or 1,8-cineole) for anti-inflammatory drugs such as NSAIDs may facilitate the distribution of drugs into gingival tissues after topical application.
- assessing the ability of TTO to penetrate the gingival tissues is warranted by the use of gas/liquid chromatography-mass spectroscopy (GC/LC-MS) of gingival biopsies. Histologic and immunofluorescence techniques could use a stain or marker (monoclonal antibody) to show the level of penetration and possible aggregation in tissue compartments.

Conclusion

TTO-containing gel applied topically to severely inflamed gingival tissues produced significant decreases in PBI and, to smaller degree, GI; the improvement of PBI per site was significantly greater in the TTO group than the other groups. The differences between the PBI and GI could have been due to variations between the nature of assessment (bleeding or visual) and differences in scoring systems i.e. criteria used and number of units. Considering evidence from i*n vitro* studies on TTO and the current study, TTO containing oral hygiene products have little or no effect on plaque accumulation and established plaque. With the evidence form the current study, TTO produced decreases in inflammation that were of clinical significance. TTO has the potential to be used as an over the counter product for symptomatic chronic gingivitis or as an adjunct to mechanical periodontal therapy.

This study points to a possible anti-inflammatory activity of TTO in inflamed gingival tissues occurring without an effect on plaque quantity. In order to confirm or refute these results, further studies on the use of TTO in periodontics are warranted.

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Appendices

Appendix I First newspaper advertisement

Do you have bad gum disease?
Non-smoker?
Join a tooth-paste trial; we'll give you **$50** & a free scale & clean.

8303 3437 Tue/Thu

Appendix II Second newspaper advertisement

New product for severe gum disease

$50 + FREE scale / clean

Do you have severe gum disease? Loose teeth? Bleeding gums?

You may be eligible for our trial.

Ph: 8303 3437 Tue & Thurs

No smokers or individuals with severe medical conditions

Appendix III Flyer advertising study to potential subjects distributed in Adelaide Dental Hospital and satellite clinics.

Do you have really bad gum disease?

If you do, join our trial & get a FREE scale and clean AND we'll give you $50

We are seeking volunteers with severe forms of gum disease (red, swollen, puffy gums that bleed a lot) to take part in a trial of a new antiseptic gel in the treatment of gum diseases.

The trial involves 3 visits for a dental examination over an 8 week period. At the final appointment, your teeth will be scaled and cleaned and you will receive $50.

Please contact us to make a screening appointment to see if you meet the entry criteria to our study.

Colgate Australian Clinical Dental Research Centre
2nd floor Adelaide Dental Hospital
Frome Road, Adelaide

8303 3437

We are unable to accept you if you are a smoker, if you have diabetes or severe heart disease, or are taking a lot of medications.

Appendix IV Flyer advertising study to dentists distributed in Adelaide Dental Hospital and satellite clinics

WANTED

Subjects for clinical trial

A clinical trial investigating the effects of a new antiseptic gel on the periodontal tissues of individuals with moderate to severe chronic gingivitis and periodontitis is starting in the Colgate Australian Clinical Dental Research Centre, Adelaide Dental Hospital.

Once included in the study, subjects attend three appointments over 8 weeks for a dental examination.

On the last appointment, subjects will receive a scale and clean and **$50**

Entry criteria:

- Females and males, aged 18-60 with
- at least 20 teeth
- Moderate to severe chronic gingivitis or periodontitis (ie severely swollen, reddened gingivae, lots of bleeding on brush ing/ probing).

Exclusion criteria

- Smokers
- People on prolonged medications such as Warfarin, antibiotic therapy, taking steroids,
- People who require antibiotic cover,
- People with pacemakers, hepatic disease, diabetes, kidney disease,
- People who are pregnant or lactating,
- People who have had periodontal therapy or antibiotics within the last 6 months.

If you have suitable patients who are willing to participate in the study, please give them a copy of the advertisement (copies supplied with this memo).

Screening appointments for patients can be made by calling Lisa at the Colgate Australian Clinical Dental Research Centre on 8303 3437.

Thank you for your assistance; if you have any queries about this trial, please contact me directly on 0403 072 058.

Dr Steven Soukoulis MDS Graduate Student Periodontics

Appendix V Patient details form used during screening of subjects

TTO CLINICAL STUDY

MEDICAL HISTORY

SURNAME.....

TITLE (Mrs, Mr, Miss)..... GIVEN NAME.....

STREET NAME AND NUMBER.....

SUBURB..... POSTCODE.....

HOME..... WORK.....

D.O.B.....

Have you ever stayed in hospital or had an operation? Yes/No

(If yes please indicate below)

.....

.....

Have you ever had any type of heart disease, high blood pressure or Rheumatic fever, heart murmur?

Yes/No

(If yes please indicate below)

.....

.....

Do you have diabetes? Yes/No

Have you ever had/have tuberculosis, asthma or other lung disease? Yes/No

Have you ever had hepatitis or other liver disease? Yes/No

Have you ever had/have kidney problems? Yes/No

Have you ever had/have bleeding problems or disorders? Yes/No

Are you pregnant? Yes/No

Do you have you any other medical problems? Yes/No

(If yes please indicate below)

.....

.....

Are you currently taking any medication?

(If yes please indicate below)

.....

.....

....

Signature of subject Date

Appendix VI Information sheet outlining the aims and protocols of the study for subjects

The University of Adelaide
Dental School

Information sheet for participants in the research project

"Effects of a tea tree oil-containing gel on chronic gingivitis."

Purpose of this study

This study has been designed to test the long term effects of a newly formulated gel containing the (anti-bacterial agent?) **tea tree oil.** We want to find out if tea tree oil-containing gels are effective in reducing the clinical signs and symptoms of chronic gingivitis.

To do this, we will need to examine the health of your gums when you brush with **one** of the following formulations: tea tree oil gel, a gel with no active tea tree oil, chlorhexidine gel (antiseptic gel).

What is involved?

At the first visit, the level of plaque, gum disease and amount of staining on teeth will be recorded. Photographs of your gums will also be taken. You will be issued with one of the preparations listed above, instructed in their use together with written instructions. You will also receive detailed instruction on oral hygiene procedures that need to be carried out during the course of the study.

The study will be conducted over a 6 week period involving a total of three visits. You will be given a new toothbrush at the start of the study, and at Week 3. You will be asked to brush using one of the gels (as instructed on the first visit) for 6 weeks.

At each of next 2 visits (Weeks 3 and 6), a review of the gum disease and plaque levels will be carried out again as will more photographs to assess progress.

On the last visit, your teeth will be cleaned and polished.

The appointment at Week 1 will take approximately 45 minutes, the Week 3 appointment 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 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?

Tea tree oil has been shown to produce no adverse side effects when used topically in the mouth.
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.

Appendix VII Consent form

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 GEL ON MODERATE TO SEVERE PERIODONTITIS

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

   EFFECTS OF A TEA TREE OIL-CONTAINING GEL 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**.....

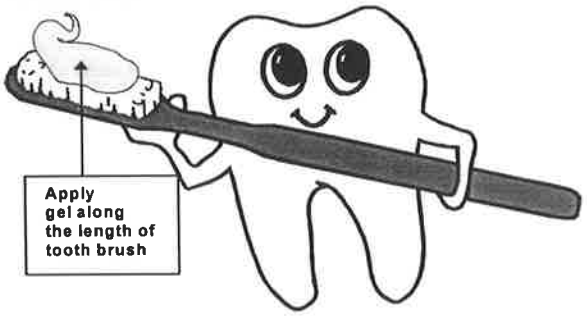
STATUS IN PROJECT .....

Appendix VIII Pamphlet with brief instructions on the use of the gel and brushing technique

Please do not use any other mouthwashes or toothpastes during the study.

HOW TO USE THE GEL

Brush with the gel **twice a day**, preferably after meals.
Put the gel along the full length of the toothbrush.



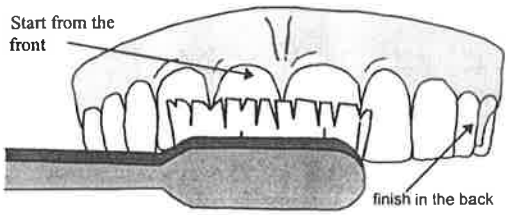
- ***Brush with the gel for at least 2 minutes (use a watch to keep track of time).***
- ***Once you have finished brushing, spit out the excess gel but do not rinse your mouth, drink or eat for 20 minutes afterwards.***

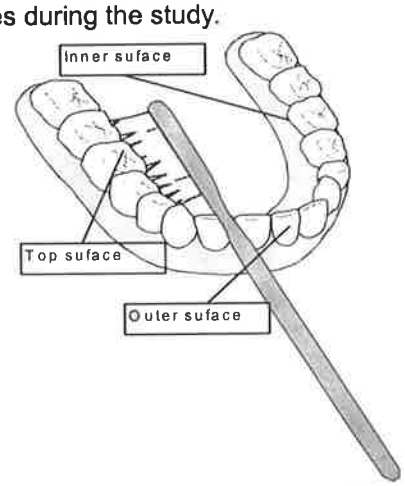
Brushing with the gel

Clean all surfaces of your teeth and gums with the gel. Your teeth have three surfaces;

- the *outer surface*, (next to your lips),
- the *inner surface*, (next to your tongue or palate) and
- the *top surface* of the teeth.

Start brushing from the outer tooth surfaces, working from the front towards the back teeth.





- Then brush the tops of the teeth, and finally the inner surfaces.
- Gently massage the gel into the gums.
- Use a **circular motion** to brush the gums and teeth, starting from the gums and moving towards the tips of your teeth.

Make sure that the gel makes contact with all gum surfaces when you brush.

Please remember not to use any other mouthwashes or toothpastes during the study.

If your health status changes during the trial (for example, pregnancy and/or you need to take antibiotics or other medication) please inform the dentist.

If you have any queries or questions please feel free to contact me, Dr Steven Soukoulis on xxxxxxxx.

Appendix IX Data collection forms

Surname:

First name:

Buccal

Lingual

Subject code ☐

Age ☐

Sex ☐ M = 1 F = 2

Formulation # ☐

Name Date

Subject code Formulation Week

Gingival index (0, 1, 2, 3)

Buccal															
	17	16	15	14	13	12	11	21	22	23	24	25	26	27	
palatal															

Buccal															
	47	46	45	44	43	42	41	31	32	33	34	35	36	37	
lingual															

Plaque Score (0, 1, 2, 3, 4, 5)

Buccal															
	17	16	15	14	13	12	11	21	22	23	24	25	26	27	
palatal															

Buccal															
	47	46	45	44	43	42	41	31	32	33	34	35	36	37	
lingual															

Papillary bleeding index (0, 1, 2, 3, 4)

Buccal															
		17/16	16/15	15/14	14/13	13/12	12/11	11/21	21/22	22/23	23/24	24/25	25/26	26/27	
palatal															

Buccal															
		47/46	46/45	45/44	44/43	43/42	42/41	41/31	31/32	32/33	33/34	34/35	35/36	36/37	
lingual															

Probing depths and periodontal attachment loss
Name

Subject code **Formulation**

Tooth	17				16				15			
Surface	DB	B	MB	P	DB	B	MB	P	DB	B	MB	P
PD												
PAL												

Tooth	21				22				23			
Surface	DB	B	MB	P	DB	B	MB	P	DB	B	MB	P
PD												
PAL												

Tooth	37				36				35			
Surface	DB	B	MB	L	DB	B	MB	L	DB	B	MB	L
PD												
PAL												

Tooth	41				42				43			
Surface	DB	B	MB	L	DB	B	MB	L	DB	B	MB	L
PD												
PAL												

Taste ☐ **1, 2, 3, 4.**