COMPARISON OF AN ESSENTIAL OIL MOUTH RINSE AND CHLORHEXIDINE ON 4-DAY INTERPROXIMAL PLAQUE REGROWTH

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KEY WORDS

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Listerine

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Clinical trial

SUMMARY

Introduction

Dental plaque is the most important etiological factor of periodontal diseases. Mechanical plaque control is the most effective way in preventing periodontal diseases. Chemical plaque control methods (such as mouthrinses) have been recommended because of some drawbacks in the mechanical methods in some areas of the dentition (such as interproximal areas). But are these mouthrinses really effective in those areas?

Aim

The aim of this study was to compare the effectiveness of an Essential Oil mouthrinse (Listerine®) on plaque formation in interproximal areas of the dentition with Chlorhexidine and Sterile water.

Materials & Methods

The study was an observer-blind, 4-day plaque regrowth, cross over study. Sixty (60) dental students volunteered to participate in the study. They received a base line scaling and polishing then used one mouthrinse for 4 days as the only oral hygiene method and then plaque index (PI) was scored using Sillness & Lõe plaque index. After that they returned to their normal oral hygiene methods for 2 weeks. Following these 2 weeks the second period of the study was done in the same way as the first. The only difference was

that they used another mouthrinse. Again after the 4-day period, plaque index (PI) was scored using Sillness & Lõe plaque index. Then they returned to their normal oral hygiene methods for 2 weeks. After the 2 weeks the third period of the study was done in the same way of the first and second periods. The only difference was that they used another mouthrinse which was different from the first and second one. Again after the 4-days period plaque index (PI) was scored using Sillness & Lõe plaque index. Then the mean plaque index was measured for each participant when using each of the mouthrinses. Also the % of plaque free surfaces and the % of interproximal plaque free surfaces were recorded for each mouthrinse.

Results

The lowest mean plaque index was recorded for Chlorhexidine (0.54). The second best anti-plaque agent was Listerine, recording a mean plaque index of 0.95, and the least effective anti-plaque mouthrinse was sterile water, with a score of 1.54.

The highest % of plaque-free surfaces was obtained with the Chlorhexidine mouthrinse (54%), whilst Listerine produced 25% plaque-free surfaces, and sterile water produced 13%.

The highest Interproximal plaque-free surfaces were obtained with the Chlorhexidine mouthrinse which was 42%, while Listerine produced 22% interproximal plaque-free surfaces and sterile water only produced 9%.

Conclusion

Analysis of variance and construction of 95% confidence intervals showed that both Chlorhexidine and Listerine significantly reduced plaque compared to the sterile water. In this study Listerine proved to be less effective than Chlorhexidine as an anti-plaque agent. However it may still be used as an adjunctive anti-plaque mouthrinse with brushing and flossing.

DECLARATION

I hereby declare that "*Comparison of an Essential Oil mouth rinse and Chlorhexidine on 4-day interproximal plaque regrowth"* is my own work, that it has not been submitted before for any degree or examination in any university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Ahmed Jarrar

October 2006

Signed:……

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DEDICATION

To my mother and father for their constant support and sacrifice.

To my supervisor whose guidance, encouragement, help and support made this project possible.

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LIST OF ABBREVIATIONS

No. **= Number**

Std. **= Standard**

Sig. **= Significant**

PI **= Plaque Index**

PFS **= Plaque Free Surfaces**

IPFS **= Interproximal Plaque Free Surfaces**

Chx **= Chlorhexidine**

CHAPTER 1

INTRODUCTION

Dental plaque is an adherent bacterial biofilm that forms on hard and soft tissues intra-orally (Fine, 1988). Bacterial colonies form if plaque is left to accumulate on tooth surfaces. Gram positive aerobic bacteria appear first in plaque followed by gram negative anaerobic and fusiform bacteria. Plaque matures and increases in pathogenicity with time (Wilkins, 1999, Fine, 1988). Mature plaque, if not removed, will result in the establishment of gingivitis after 2-3 weeks (Theilde *et al,* 1966, Lőe *et al,* 1965).

Mechanical plaque control has been regarded as the most effective method of plaque removal which will consequently prevent the establishment of gingivitis (Santos, 2003). This method of plaque control is not 100% effective and some residual plaque is frequently left behind after brushing and flossing (DePaola *et al,* 1989). Even in well trained and educated patients their compliance for daily brushing and flossing diminishes with time (Stewart and Wolfe, 1989). Interdental plaque is not effectively removed by brushing. It has been estimated that approximately 10% of the population floss regularly and effectively (Kalsbeek *et al,* 2000, Macgregor *et al,* 1998, Stewart *et al,* 1997, Ronis *et al,* 1993).

Chemotheraputic agents (e.g. mouthrinses) are recommended for use as adjuncts to mechanical plaque control (Bouwsma, 1996, Wolff, 1985); these are proposed to be

effective specifically for interdental areas. Essential oil mouthrinses (such as Listerine®) and Bis-biguanide mouthrinses (such as Chlorhexidine) have been accepted as adjuncts to mechanical cleaning by the American Dental Association (ADA) (Council on Dental Therapeutics, 1988).

The efficacy of mouthrinses as adjuncts to mechanical methods of cleaning is determined by their ability to produce plaque-free interproximal surfaces. Whilst Listerine® has been recommended as an effective anti-plaque agent specifically to reduce interdental plaque accumulation, its usefulness has not been determined (Okamoto *et al,* 1988, Axelsson and Lindhe, 1978, Cumming and Lőe, 1973, Lovdal *et al,* 1961). The efficacy of Chlorhexidine is well documented and it is considered to be the most effective anti-plaque agent available (i.e. "the gold standard") (Overholser, 1990, Banting *et al*, 1989, Sergeto *et al,* 1986, Grossman *et al,* 1986, Lang *et al,* 1982, Loe *et al,* 1976, Flotra *et al,* 1972, Lőe and Schiott, 1970).

The aim of this study is to determine the effectiveness of an essential oil mouthrinse (Listerine®) on 4-day interproximal plaque regrowth compared to Chlorhexidine and Sterile Water.

CHAPTER 2

LITERATURE REVIEW

2.1- Introduction

Dental plaque is the main etiological factor of periodontal diseases.

Many types of mouthrinses are available and are claimed to have anti-plaque effects. Of these, only 2 (Listerine® and Peridex®) have been accepted by the American Dental Association. But are they really effective?

This chapter will focus on plaque formation and how to prevent its formation mechanically and chemically. The properties of Listerine® and Peridex® are discussed.

2.2- Dental plaque

2.2.1- Definition

Dental plaque is a complex of several hundred species of bacteria living together, forming an adherent biofilm. It is the principal etiological factor in periodontal diseases and caries (Fine, 1988). If plaque is allowed to accumulate, with no intervention or oral hygiene methods, gingivitis is established after 2-3 weeks of plaque formation (Theilade *et al,* 1966, Lőe *et al,* 1965).

2.2.2- Supragingival plaque formation

Plaque formation undergoes 3 stages as follows:

Stage 1- The acquired pellicle is formed as an acellular coating composed of salivary proteins, on clean tooth surfaces. This layer enhances the adhesion of bacteria to the teeth.

Stage 2- Within a few hours, gram positive aerobic bacteria, mainly cocci (the initial colonizers) adhere to the pellicle. Following this initial colonization, bacterial growth and multiplication increases rapidly. After 2-4 days more gram negative anaerobic (filamentous and cocci) organisms and fusiform bacteria with higher pathogenicity begin to colonize.

Stage 3- Plaque maturation occurs after day 4. The predominant organisms are filamentous with some spiral and spirochete species (Wilkins, 1999, Listgarten, 1999, Fine, 1988).

2.2.3- Sub gingival plaque and calculus formation

Bacterial products that pass through the junctional epithilium cause the inflammatory changes in the teeth supporting tissues. This initiates gingivitis (Page, 1991). These changes facilitate bacterial colonization in the sub-gingival tissues.

Sub-gingival plaque is composed of predominantly gram negative anaerobic organisms (Bernimoulin, 2003). Tannerella forsythis, Porphyromonas gingivalis and Treponema denticola are considered to be the most predominant organisms in sub gingival plaque and these bacteria are causative in the establishment of periodontitis (Socransky and Haffajee; 2002).

If plaque is allowed to accumulate it will become mineralized and form calculus. The establishment of calculus results in further bacterial accumulation as a result of its porous nature and rough surface. Consequent to the formation of calculus periodontal disease is established and progressive loss of attachment may follow (Bernimoulin, 2003, Wilkins, 1999, DePaola *et al,* 1989).

2.3- Incidence of periodontal disease

Periodontal disease is one of the most important concerns for dentists and patients (Ciancio, 2003).

Morris *et al* (2001) reported that in the United Kingdom 40-45% of adults have moderate destructive periodontal disease and 5-10% have a severe form of the disease. They also reported that 72% of adults have visible plaque; which is the main causative factor of periodontal disease.

In the United States 50% of adults have gingivitis affecting at least 3-4 teeth, two-thirds of the population have sub gingival calculus, and about a third have periodontitis (Oliver *et al*, 1998).

Albandar and Rams in 2002 reported that more than 82% of the United States adolescents have overt gingivitis and signs of gingival bleeding. They reported that the prevalence of gingivitis for children and adolescents in other parts of the world is almost the same or possibly higher than that of the United States adolescents.

These authors in their report further suggested that improving the oral hygiene of the population will have a great impact on the occurrence of periodontal disease. This suggestion had been expounded by Morris *et al* (2001) who also suggested that improved oral hygiene would result in the widespread improvement in management of the disease.

2.4- Mechanical plaque control

2.4.1- Introduction

Mechanical control of plaque, i.e. brushing and flossing is considered to be the most effective method of prevention of periodontal disease in spite of some shortcomings (Santos, 2003).

2.4.2- Short comings of mechanical methods

Tooth brushing and flossing are difficult methods of plaque control for young patients and for persons who have manual dexterity limitations. Consequently, even with diligent effort plaque is seldom removed completely, using these methods only (Baker, 1993, Ciancio, 1988).

Well-educated and motivated patients achieve the best results of plaque control and this minimizes periodontal diseases (Axelsson and Lindhe, 1987). Even in these motivated patients, compliance diminishes over time (Stewart and Wolfe, 1989, Axelsson and Lindhe, 1987).

Patients who brush regularly, frequently consider flossing interdental areas difficult and time-consuming. Continuous motivation and patient compliance is essential to establish a good plaque control programme (Bouwsma, 1996, Wolff, 1985). Several studies indicate that only between 2-10% of the population perform interdental hygiene (floss or toothpicks) on a daily basis (Kalsbeek *et al,* 2000, Macgregor *et al,* 1998, Stewart *et al,* 1997, Ronis *et al,* 1993). It is estimated that only 20% of these patients floss effectively (Lang *et al,* 1994).

2.4.3- Compliance with mechanical methods

Stewart and Wolfe (1989), as well as Axelsson and Lindhe (1987) reported that patient compliance for regular mechanical oral hygiene diminished with time. Compliance for daily flossing ranges from 10-40% (Craig and Montague, 1976, Nixon, 1978, Bakdash, 1995).

2.4.4- Conclusion

As a result of poor patient compliance with mechanical methods of plaque control, it is important to find adjunctive methods which need less effort but have proven antimicrobial activity (Brown *et al,* 1996, DePaola *et al,* 1989). Several reports

indicate that chemical control of plaque may be an effective and useful mean to overcome these shortfalls (Yengopal, 2004, Bouwsma, 1996, Wolff, 1985).

2.5- Chemical plaque control

2.5.1- Introduction

Antiseptics are chemical agents that will either kill microorganisms (Ciancio, 2000) or interfere with the colonization of the tooth surfaces (Fine *et al,* 1996). These active agents are effective as anti-plaque and anti-gingivitis mouthrinses (Ciancio, 2000). Chlorhexidine, essential oils, triclosan, iodine and cetylpyridinium chloride are the most common antiseptics in use (Ciancio, 2003). They are widely used as adjunctive methods to mechanical tooth cleaning (Bouwsma, 1996, Wolff, 1985).

2.5.2- Properties of an effective antiseptic

Baker (1993) suggested that the properties of an effective antiseptic should include:

- Broad spectrum antimicrobial activity against wide range of micro-organisms including gram positive, gram negative, fusobacteria and spirochetes.

- They should be safe to use and not time-consuming.

- These agents should be cost-effective, easy to use and able to reach the areas of disease initiation.

- They should be pleasant tasting and palatable.

Based on clinical trials, the American Dental Association accepted two mouthrinses as anti-plaque and anti-gingivitis agents (Cianco 2000). These mouthrinses are Peridex ® (Zila Pharmaceuticals, Phoenix, AZ, USA; Chlorhexidine, CHX) and Listerine ® (Pfizer Consumer Healthcare, Morris Plains, NJ, USA; Essential oils, EO).

2.5.3- Chlorhexidine

2.5.3.1- Composition and group

Chlorhexidine is a Bisbiguanide anti-plaque agent. This anti-plaque agent is effective at a concentration of 0,2% (Lang *et al,* 1982).

2.5.3.2- Efficacy

The efficacy of Chlorhexidine mouthrinse is well-documented. It is the most effective anti-plaque/anti-gingivitis agent and is considered to be the "gold standard" (Overholser, 1990, Banting *et al,* 1989, Sergeto *et al,* 1986, Grossman *et al,* 1986, Lang *et al,* 1982, Lőe *et al,* 1976, Flotra *et al,* 1972, Lőe and Schiott, 1970).

2.5.3.3- Advantages

Bacterial pathogenicity is not increased with prolonged use of Chlorhexidine (Briner *et al,* 1986, Emilson and Fornell, 1976, Schiott *et al,* 1976).

Microbial resistance does not increase with long term usage (Minah *et al,* 1989).

This Bisbiguanide has a broad antimicrobial range (DePaola *et al,* 1996, Jenkins *et al*, 1994, Kubert *et al, 1993*)

Chlorhexidine is able to penetrate plaque and kill the bacteria in deeper layers of the biofilm (Pan *et al,* 2000, Fine *et al,* 1996).

Chlorhexidine has the affinity to bind to tissues (Substantivity) and stay active for more than 12 hours (Netuschil *et al*, 1995, Weeks *et al*, 1988, Wolff, 1985, Roberts and Addy, 1981, Addy and Right, 1978, Turesky *et al,* 1977, Schiott, 1973).

2.5.3.4- Disadvantages

Taste alteration that can last up to 4 hours after rinsing with Chlorhexidine (Ciancio, 2000).

Chlorhexidine can cause staining of the tongue, teeth and restorations and also supragingival calculus (Ciancio, 2000).

Toothpaste ingredients reduce the efficacy of Chlorhexidine. Manufacturers recommend that this anti-plaque agent should be used within 30 minutes of tooth brushing .

2.5.3.5- Mechanism of action

Chlorhexidine inhibits plaque formation by binding to bacteria and preventing their adhesion to the teeth (Wolff, 1985),

This anti-plaque agent also binds to salivary mucins and therefore reduces pellicle formation (Fine *et al,* 1996, Wolff, 1985),

It is also well documented that Chlorhexidine causes a breakdown in bacterial cell membranes causing their death (Fine, 1988).

2.5.3.6- Clinical evidence of efficacy

In two controlled studies Grossman *et al* (1986) and Grossman *et al* (1989) compared the effects of rinsing with 0.12% of Chlorhexidine with a placebo for a period of 6 months. In the first study of 380 patients they reported that Chlorhexidine significantly reduced plaque by 61% and gingivitis by 49% ($P < 0.05$). In the second study of 481 patients they reported that Chlorhexidine significantly reduced plaque by 49% and gingivitis by 31%.

2.5.4- Listerine® 2002.

2.5.4.1- Composition and group

Listerine belongs to the phenolic group of anti-plaque agents and is composed of 4 essential oils as active ingredients. These oils are Thymol 0.064%, Eucalyptol (0.092%), Methyl salicylate (0.060%) and Menthol (0.042%) . These active agents are solubilized in alcohol medium (manufacturer's composition).

2.5.4.2- Advantages

When Listerine is used for a long period, the bacterial pathogenicity does not increase (Minah *et al,* 1989, Walker *et al,* 1989). No antimicrobial resistance was noticed after long-term use of Listerine (Minah *et al,* 1989).

Listerine has been reported to have a broad antimicrobial range in several studies (DePaola *et al,* 1996, Jenkins *et al*, 1994, Kubert *et al,* 1993, Ross *et al,* 1989, Pitts *et al,* 1983).

Listerine is able to penetrate plaque and kill the bacteria in deeper layers of the biofilm (Pan *et al,* 2000, Fine *et al,* 1996).

Listerine binds to tissues and stay active for several hours (Fine *et al,* 2001, Ross *et al,* 1989, DePaola, 1989). This property increases and prolongs the anti-plaque activity of this agent.

Listerine does not stain the tongue, teeth and restorations (Charles *et al,* 2001, Overholser *et al,* 1990, DePaola *et al,* 1989, Gordon *et al,* 1985, Lamster *et al,* 1983).

Listerine does not change taste perception and there is no increased calculus formation (Charles *et al,* 2001, Overholser *et al*, 1990).

Listerine can be used immediately after brushing. The efficacy of Listerine is not decreased by the consistuent chemicals of toothpaste (Santos, 2003).

2.5.4.3- Mechanism of action

Listerine initially kills the superficial bacteria then it kills the bacteria in the deeper layers of the biofilm (Pan *et al,* 2000).

The mechanisms by which Listerine kills bacteria include the following:

Disruption of bacterial cell walls which results in lysis of the cellular structures. Listerine is also known to inhibit bacterial enzymatic activity (Kubert *et al,* 1993, Fine, 1988). A mechanism which has been reported by Dennison *et al* (1995) indicated that this essential oil results in the extraction of bacterial endotoxins with the resultant reduction in the pathogenicity of plaque.

2.5.4.4- Clinical evidence of effectiveness

In a double blinded, placebo controlled clinical study Gordon *et al* (1985) reported that Listerine reduced plaque formation by 19.5% and gingivitis formation by 23.5%.

In a similar study Lamster *et al* (1983) reported that Listerine reduced plaque formation by 22.2% and gingivitis formation by 28.3%

DePaola *et al* (1989) tested the efficacy of Listerine and they reported a reduction in plaque and gingivitis formation by 34%.

Charles *et al* (2000) in a randomized, observer blinded, cross-over clinical trial studied the bacterial counts from interproximal surfaces after using Listerine as adjunct to oral hygiene measures. They found that plaque reduction was 43.8% as compared with the control.

The findings of all these studies quoted above were statistically significant.

2.5.4.5- Other uses for Listerine

This essential oil has been recommended for use after periodontal surgery since it reduces plaque formation without interfering with the healing process (Laspisa *et al,* 1994, Zambon *et al,* 1989).

Essential oil mouthrinses are also effective against Streptococcus mutans which is one of the most important causative bacteria for dental caries (Fine *et al,* 2000).

Essential oil mouthrinses also reduces plaque and inflammation around dental implants (Ciancio *et al,* 1995).

Listerine can be used effectively in the management of halitosis with no side effects even if used for a long period (Yengopal, 2004).

Essential oil mouthrinses can also be used as a pre-procedural mouthrinse. This will reduce the numbers of bacteria in the aerosols produced by ultrasonic scaling (Fine *et al,* 1993).

Rinsing and sub-gingival irrigation with essential oil mouthrinses before dental procedures will reduce chances of bacteraemia that might result from dental procedures (Fine *et al,* 1996).

2.5.4.6- Controversies about alcohol (ethanol) used in Listerine

Alcohol (ethanol) is used in Listerine mouthrinse as a vehicle to solubilize the oil components (Ciancio *et al,* 1995, Lamster *et al,* 1983). Alcohol has no beneficial effect on reducing plaque and gingivitis (Gordon et al, 1985, Lamster *et al,* 1983).

Some carcinogens found in alcoholic beverages are associated with cancer (Ciancio, 1993), but ethanol which is used in mouthrinses is free of these carcinogens (Claffey, 2003, Ciancio, 1993). Studies which examined the association of using alcohol-containing mouthrinses and oral cancer concluded that thre was no significant evidence of a causal relationship (Winn *et al,* 2001, Elmore and Horwitz, 1995).

2.5.5- Comparative studies of Chlorhexidine and Listerine

Both Chlorhexidine and Listerine® have broad antimicrobial range (DePaola *et al,* 1996, Jenkins *et al*, 1994, Pitts *et al,* 1983, Kubert *et al,* 1993, Ross *et al,* 1989). They have the ability to penetrate and kill the bacteria even in the deeper layers of the bacterial plaque (Pan *et al,* 2000, Fine *et al,* 1996). Chlorhexidine has the ability to penetrate and be effective at deeper plaque layers (Netuschil *et al*, 1995).

Chlorhexidine demonstrates good substantivity (the affinity to bind to tissues and stay active). Chlorhexidine may stay active for more than 12 hours (Netuschil *et al*, 1995, Weeks *et al*, 1988, Wolff, 1985, Roberts and Addy, 1981, Addy and Right, 1978, Turesky *et al,* 1977, Schiott, 1973). The substantivity of Listerine is less than that of Chlorhexidine (Fine *et al,* 2001, Ross *et al,* 1989, DePaola, 1989).

In a six month randomized, double blind clinical trial Overholser *et al* (1990) compared the effects of Listerine and Chlorhexidine. They reported that Listerine inhibited 36.1% of plaque formation, while Chlorhexidine inhibited plaque formation by 50.3%. The results for both agents were statistically significant. They also reported that Listerine inhibited 35.9% of gingivitis formation , Chlorhexidine which inhibited gingivitis formation by 30.5%. Both results were statistically significant.

Listerine does not stain tooth surfaces (Charles *et al,* 2001, Overholser *et al,* 1990, DePaola *et al,* 1989, Gordon *et al,* 1985, Lamster *et al,* 1983). Also Listerine does not cause taste alterations nor does it increase calculus formation (Charles *et al,* 2001, Overholser *et al*, 1990). On the contrary Chlorhexidine causes taste alterations that can last up to 4 hours after rinsing. A further disadvantage is that the tongue, teeth

and restorations as well as supragingival calculus stain after prolonged use (Ciancio 2000). Chlorhexidine should only be used 30 minutes after tooth brushing because tooth paste ingredients reduce its efficacy (Peridex 2001). Listerine, however, can be used immediately after teeth brushing without a reduction in its efficacy (Santos 2003).

Chlorhexidine and Listerine can penetrate plaque and kill bacteria in the deeper layers of plaque. They have a broad antimicrobial range that makes them much more effective than other antiseptics (Bernimoulin, 2003).

The efficacy of Chlorhexidine and Listerine have been reported separately . Although these studies have some differences in their methodology, they mainly compared the use of either Chlorhexidine or Listerine with a placebo. The findings regarding the reduction in plaque formation and gingivitis varied in several studies (Charles *et al,* 2001, Overholser *et al,* 1990, DePaola *et al,* 1989, Grossman *et al,* 1989, Grossman *et al,* 1986, Gordon *et al,* 1985, Lamster *et al,* 1983, Lang *et al,* 1982, Lőe *et al,* 1976). Review of the literature failed to identify any study which compared the effectiveness of Chlorhexidine and Listerine on interproximal plaque growth.

2.5.6- Clinical evidence of Listerine as an effective adjunctive mouth rinse

Bauroth *et al* (2003) in a six month, randomized, observer blinded, and clinical trial compared the effects of using dental floss versus rinsing with an essential oil mouthrinse (Listerine®) on interproximal areas. They concluded that the twice daily use of Listerine in conjunction with professional care (prophylaxis) and tooth

brushing was as good as flossing daily in conjunction with tooth brushing, in reducing interproximal plaque and gingivitis. The results of both flossing and rinsing with Listerine were statistically significant in plaque and gingivitis reduction when compared to the negative control group that used brushing only. And they recommended the use of essential oil mouthrinses as adjunctive to the mechanical oral hygiene regimens.

Sharma *et al* (2002) conducted a study in which they compared the effects of using dental floss versus rinsing with essential oils (Listerine) mouthrinse on interproximal areas. The results of this study were similar to those of Bauroth *et al* 2003.

Both studies concluded that twice daily use of Listerine was as good as flossing once daily in reducing interproximal plaque and gingivitis. Yengopal, (2004) reported similar findings.

In another randomized controlled trial by Sharma *et al* (2004) it was concluded that for patients with gingivitis who brush and floss, the adjunctive use of an essential oil mouthrinse will provide a clinically significant reduction of plaque and gingivitis and that the use of such a containing mouthrinse is beneficial.

2.5.7- Conclusion

Several studies have proved that Chlorhexidine and Listerine are effective adjuncts to mechanical methods of tooth cleaning (Okamoto *et al*, 1988, Axelsson and Lindhe, 1978, Cumming and Loe, 1973, Lovdal *et al,* 1961). In patients who have difficulties

in practicing mechanical methods of plaque control, these adjunctive anti-plaque anti-gingivitis mouthrinses have great advantages.

The efficacy of Chlorhexidine is well documented and it is considered to be the most effective anti-plaque agent available (Overholser, 1990, Banting *et al*, 1989, Sergeto *et al,* 1986, Grossman *et al,* 1986, Lang *et al,* 1982, Loe *et al,* 1976, Flotra *et al,* 1972, Lőe and Schiott, 1970). Unfortunately the efficacy of Listerine has not been specifically determined (Okamoto *et al,* 1988, Axelsson and Lindhe, 1978, Cumming and Lõe, 1973, Lovdal *et al,* 1961). **Review of the literature failed to reveal a randomized controlled observer, three period cross-over clinical trial which compare the efficacy of Chlorhexidine versus Listerine®** . The aim of this study is to determine the efficacy of (Listerine®) on 4-day interproximal plaque regrowth compared to Chlorhexidine and Sterile Water.

CHAPTER 3

AIM AND OBJECTIVES

3.1 – Aim of the study

The aim of this study was to compare the effect of an essential oil mouthrinse on 4-day interproximal plaque regrowth with Chlorhexidine and Sterile Water.

3.2 – Objectives of the study

1- To determine the percentage of interproximal plaque-free surfaces produced after using Listerine mouthrinse as the only oral hygiene measure for 4 days as compared to Chlorhexidine and Sterile Water.

2- To determine the percentage of plaque-free surfaces produced after using Listerine mouthrinse as the only oral hygiene measure for 4 days as compared to Chlorhexidine and Sterile Water.

3- To compare the Plaque Index reduction after using Listerine, Chlorhexidine and Sterile Water mouthrinses.

CHAPTER 4

MATERIALS & METHODS

4.1- Study design

The study was a randomized controlled, observer-blinded, three period cross-over, clinical trial.

4.2- Study population

Dental students of the faculty of dentistry at the University Western Cape.

4.3- Sample size

60 students aged 16-34 years participated in the study. The size of the sample was verified with the aid of a statistician.

4.4- Sampling procedure

The participants were distributed into 3 groups of 20 each . Each consecutive participant was allocated to one of the three mouthrinse groups.

Group one (I) in the first period used Listerine mouthrinse, in the second period Chlorhexidine and in the third period, sterile water

Group two (II) used Chlorhexidine in the first period then sterile water in the second and Listerine mouthrinse in the third period.

Group three (III) used sterile water in the first period then Listerine in the second and Chlorhexidine in the third period.

4.5- Inclusion criteria

All subjects must have a minimum of 20 teeth with good oral hygiene and gingival health.

4.6- Exclusion criteria

The following exclusion criteria were applied:

Volunteers with fixed or removable appliances or any dental prostheses.

Volunteers with any medical or pharmacological history that could compromise the conduct of the study.

Volunteers with any oral pathology.

Volunteers that had treatment with antibiotics or anti-inflammatory drugs in the preceding 2 months.

Volunteers with probing depths more than 3mm.

4.7- Data collection

At the beginning of the study the investigator examined all volunteers and recorded complete information about their medical and dental status. The investigator explained the study in detail verbally and provided the participants with all the information in writing that was necessary for them to give informed consent regarding the study. In addition to the verbal explanation, the consent form (Appendix IV) also contains all the details of the study. Each participant was allowed the opportunity to question any information about which they were unclear. The participants were then enrolled in the study after having signed a document of informed consent (Appendix IV). Each participant was allocated a code to ensure anonymity.

4.7.1- The first experimental period

On day 1 of the first period the participants received a scale and polish to remove all plaque, calculus and stains. A disclosing agent was used immediately to ascertain that all plaque and calculus was removed. They were then asked to cease their usual oral hygiene procedures for the following 4 days. It was emphasized that the only means of oral hygiene that they were allowed was the use of the mouthrinse that was dispensed to them. These mouthrinses were dispensed in coded 200ml bottles that were identical for each of the three mouthrinses (see Figures 4.1, 4.2, 4.3). The dispensing of the mouthrinses was done by a nursing sister in the Department of Periodontology to ensure examiner blindness in the study. Participants were asked to rinse with 20 ml of the allocated mouthrinse twice daily for 1 minute. All instructions were given in detail verbally as well as in writing (Appendix I).

On day 5 all participants were scored for plaque using Plaque index (as described by Lőe, 1967) with some modifications to increase the predictive accuracy of the index (Appendix II-A and II-B). Plaque scoring requires light, drying of teeth and gingiva and a probe.

Figure 4.1: Listerine mouthwash

Figure 4.2: Chlorhexidine mouthwash

Figure 4.3: sterile water

The first modification was that Plaque scores were measured on 6 areas of each tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) instead of 4 areas as was suggested by Silness and Lőe.

The second modification was that a disclosing agent was used in addition to the use of the probe which increased the accuracy of the scores (Appendix II-B).

The data collected was noted on a recording sheet (Appendix III).

The mean plaque index was measured for each participant by adding the scores of the whole surfaces then dividing it by the number of surfaces examined.

A score of 0 was considered as plaque-free surface (plaque absence), while the other 3 scores (1, 2, and 3) were considered as positive scores (plaque present).

4.7.2- The first wash out period

After the end of the first period of participation, a wash out period of 2 weeks followed in which participants returned to their normal oral hygiene methods.

4.7.3- The second experimental period

After the two-week wash out period, the participants received a scale and polish to remove all plaque, calculus and stains as was determined in the first period of the experiment. They then ceased all normal oral hygiene procedures for the following 4 days (Ramberg *et al*, 1992), once again the participants were requested to use only the allocated mouthrinse as dispensed by the nursing sister in the Department of Periodontology. They were asked to use 20ml of the mouthrinse twice daily as their only means of oral hygiene during this period. On day 5 the participants were scored for plaque using the plaque index of Lőe, 1967.

4.7.4- The second wash out period

After the end of the second period, a wash out period of 2 weeks followed in which participants again returned to their normal oral hygiene methods.

4.7.5- The third experimental period

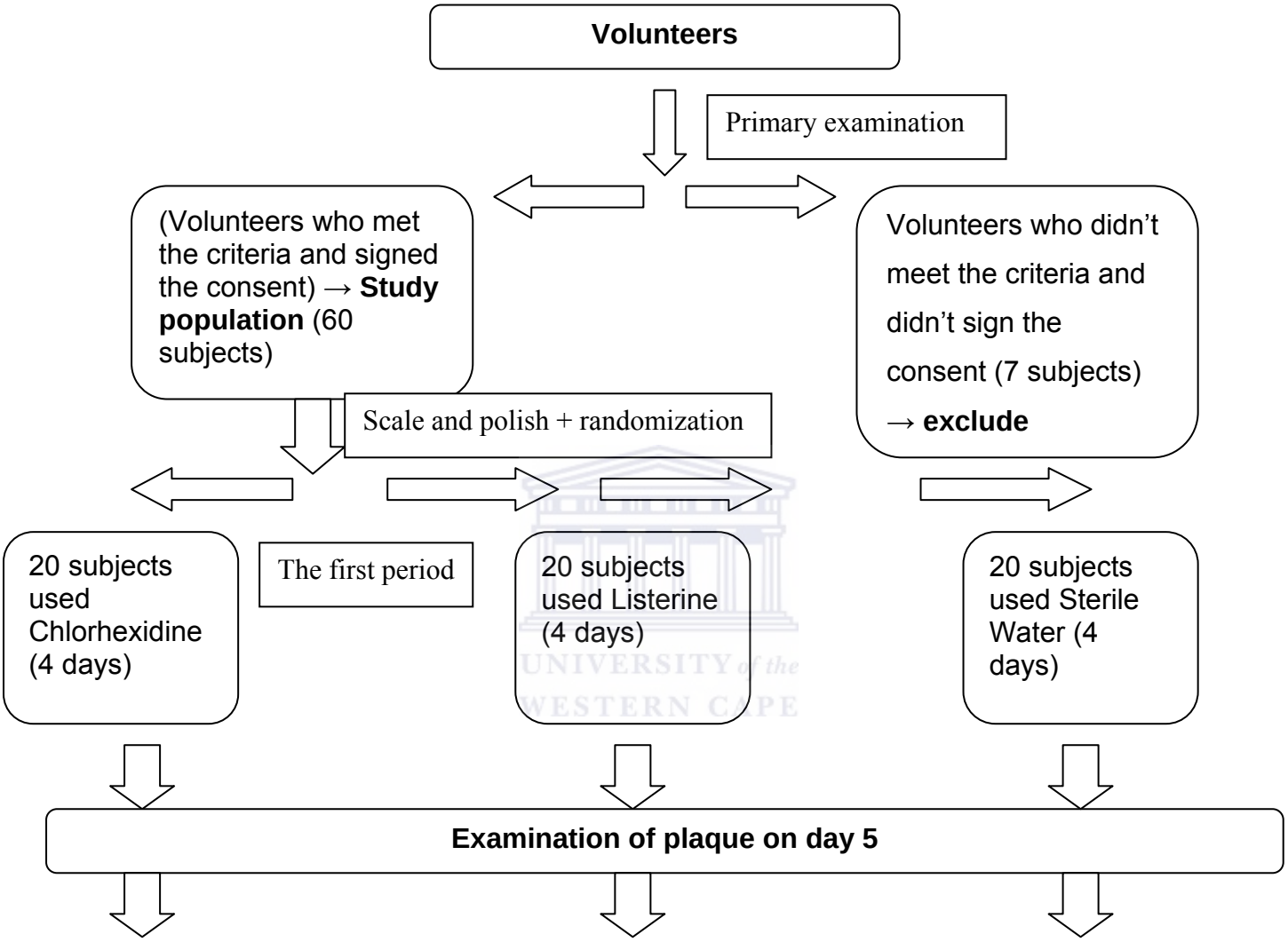
After the second wash out period (two weeks) the participants received a scale and polish to remove all plaque, calculus and stains (to reach score 0 in Silness and Lőe plaque index). Then they ceased all normal oral hygiene methods for the following 4 days except for the use of the dispensed mouthrinse. They were asked to use 20ml of the mouthrinse twice daily for 4 days and on the fifth day their plaque scores were once again recorded using the plaque index of Lőe, 1967. At the end of this final phase of the experiment all participants received a final scale and polish.

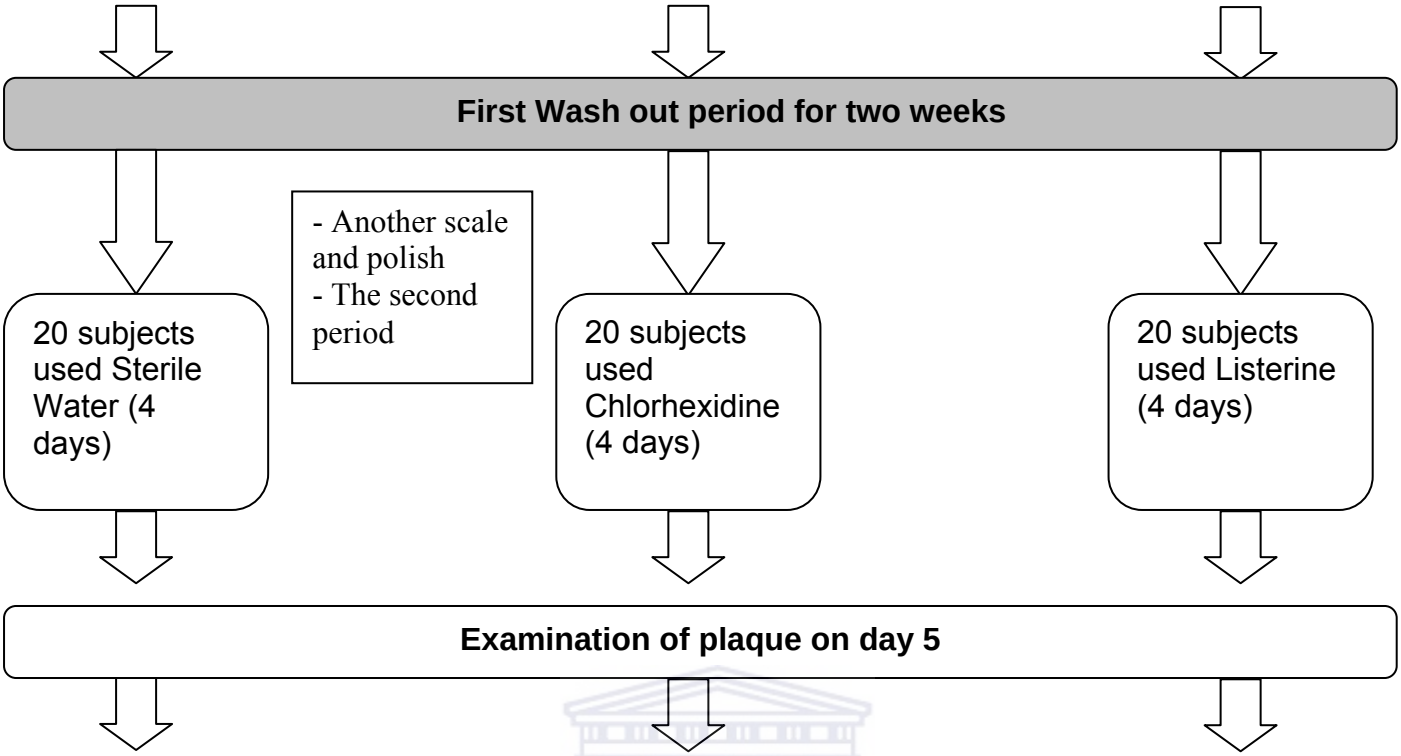
4.7.6- Intra-examiner calibration

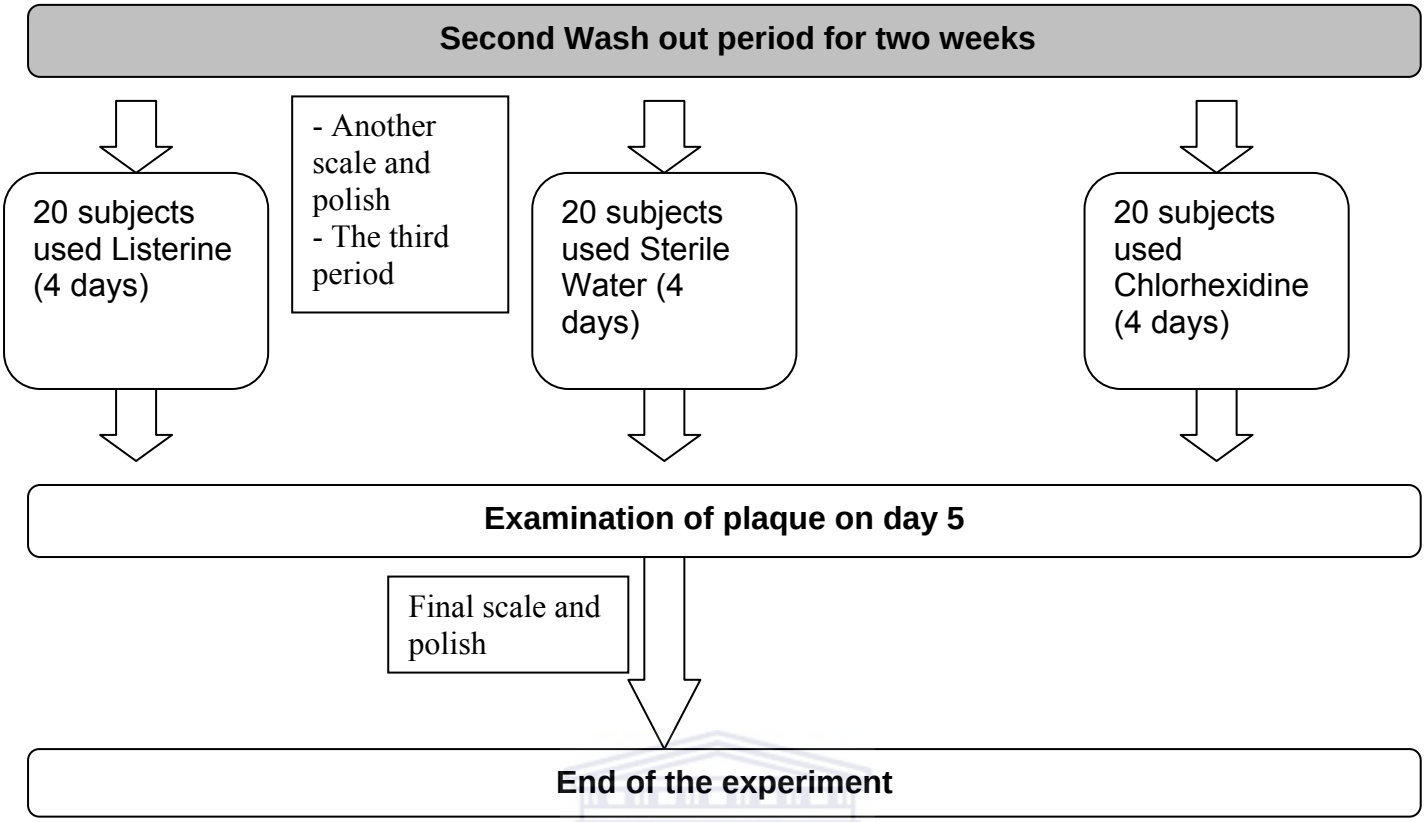
To ensure reliability of the results the examiner re-scored the PI for 20% of the sample 1 hour after the end of the first experimental period. A total of 12 participants were thus re-examined and there was no significant difference in the results obtained between the first and second examinations.

- **Organogram of the experiment:**

The following three pages outline the three phases of this experiment:







4.8- Data analysis

The data was analyzed using a commercially available statistical software package (SPSS 13.0, SPSS Inc.).

4.8.1- % of Plaque-free surfaces (whole mouth)

The % of plaque-free surfaces was calculated for each participant using the following formula:

$$\% \text{ of plaque-free surfaces} = \frac{\text{No. of surfaces with score 0}}{\text{Total nomber of surfaces}} \times 100\%$$

The mean % of plaque-free surfaces was determined for each mouthrinse.

4.8.2- % of Plaque-free interproximal surfaces

The % of plaque-free Interproximal surfaces per participant was calculated using the following formula:

% of plaque-free interproximal surfaces =

$$\frac{\text{No. of Interproximal surfaces with score 0}}{\text{Total no. of interproximal surfaces}} \times 100\%$$

The mean % of plaque-free Interproximal surfaces was determined for each mouthrinse.

4.8.3- Mean plaque index

The mean plaque index was calculated for each participant following each experimental period. This was calculated by adding the plaque scores of all the surfaces recorded and dividing it by the number of surfaces examined

The mean plaque index for each mouthrinse was calculated by combining the mean plaque index of all participants subsequent to the use of each mouthrinse (three times).

A Kruskal-Wallis analysis of variance (ANOVA) test was carried out to determine if statistically significant differences existed between the experimental groups at a significance level of $p \leq 0.05$.

CHAPTER 5

ETHICAL CONSIDERATION

1- Participation in the study was on a voluntary basis.

2- People who accepted to participate in the study were presented with a consent form to sign, in which all the details about the study were clear. (Appendix 4).

3- The consent form also addressed the confidentiality of the information obtained and its use.

CHAPTER 6

RESULTS

6.1 – Introduction

All participants (60 dental students) completed the rinsing regimens satisfactorily and without any noted side-effects. Each subject verified that they had complied with the experiment instructions.

This chapter reports the findings of this study including the plaque index, % of plaque-free surfaces, and % of interproximal plaque-free surfaces as well as the statistical analysis of the results.

6.2 – Age of participants:

60 students participated in the study with a mean age of 22 years, as shown in Table .

Statistical measurements	Mean	Median	Std. deviation	Minimum	Maximum
Age	22.4	22.0	2.965	16	34

Table 6.1: Age distribution among participants

6.3 – Gender of participants:

41 males (68%) and 19 (32%) females were randomly selected for the study.

6.4 – Mean Plaque index scores for each mouthrinse

The mean plaque index scores for each of the mouthrinses are presented in Table 6.2:

Group		Plaque index
Listerine	Mean	0.94
	Std. deviation	0.18
Chlorhexidine	Mean	0.54
	Std. deviation	0.09
Sterile water	Mean	1.54
	Std. deviation	0.09

Table 6.2: Mean plaque index scores

The lowest mean plaque score was recorded for Chlorhexidine (0.54). The second best anti-plaque agent was Listerine recording a mean plaque index score of 0.94, and the least effective anti-plaque mouthrinse was sterile water with a PI of 1.54.

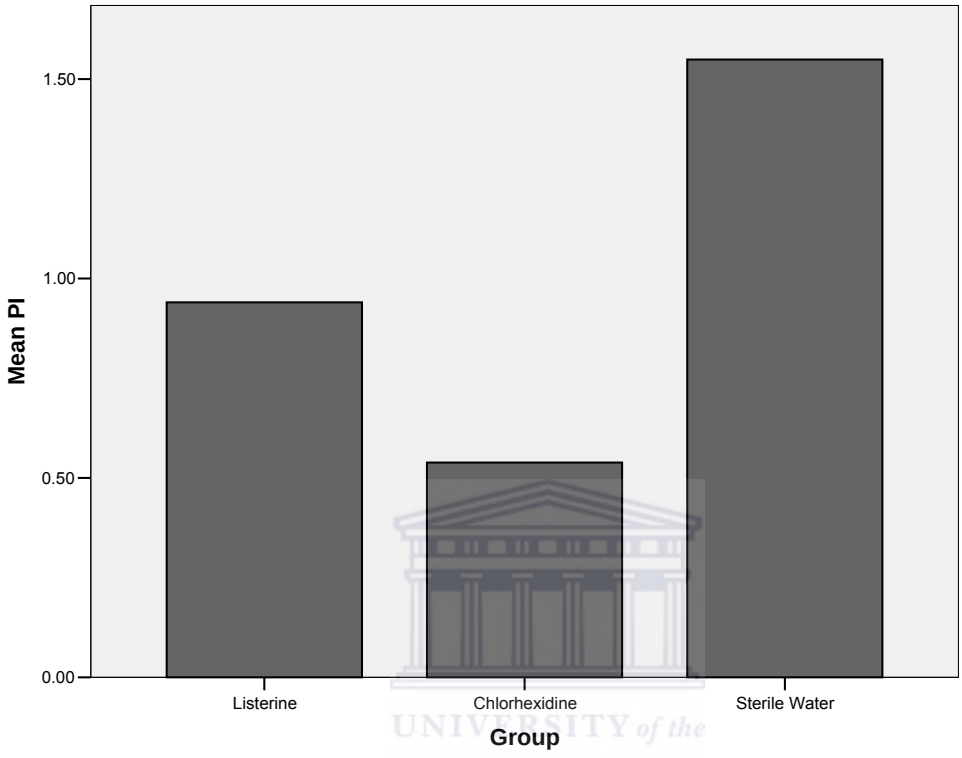


Figure 6.1: Mean plaque index scores

Figure 6.1 represents a bar-graph for the various mean plaque index scores of each experimental group in the study.

6.5 – Percentage of Plaque-Free Surfaces Scores

The highest % of plaque-free surfaces was obtained with the Chlorhexidine mouthrinse (54%), whilst Listerine only produced 25% plaque-free surfaces, and sterile water produced 13% (Table 6.3).

Group		% of Plaque-Free Surfaces
Listerine	Mean	25
	Std. deviation	4.32
Chlorhexidine	Mean	54
	Std. deviation	5.84
Sterile water	Mean	13
	Std. deviation	4.46

Table 6.3: Percentage of Plaque-Free Surfaces

6.6 – Percentage of Interproximal Plaque-Free Surfaces Scores

The highest interproximal plaque-free surfaces were obtained with the Chlorhexidine mouthrinse which was 42%, while Listerine produced 22% interproximal plaque-free surfaces and sterile water only produced 9% (Table 6.4).

Group		% of Interproximal Plaque Free Surfaces
Listerine	Mean	22
	Std. deviation	4.84
Chlorhexidine	Mean	42
	Std. deviation	5.18
Sterile water	Mean	9
	Std. deviation	3.78

Table 6.4: Percentage of Interproximal Plaque-Free Surfaces

6.7 Comparison of Mouthrinses:

A Kruskal-Wallis analysis of variance (ANOVA) test was carried out to determine if statistically significant differences existed between the experimental groups at a significance level of $p \leq 0.05$. A summary of the results is represented in Table 6.5.

		Sum of squares	df	Mean square	F	Sig.
% IPFS	Between groups	33900.144	2	16950.072	787.848	.000
	Within groups	3808.050	177	21.514		
	Total	37708.194	179			

Table 6.5: ANOVA results (overall comparison of groups)

The results of the Kruskal-Wallis test showed that there was a highly significant difference amongst the mouthrinses groups tested ($p \leq 0.05$). Once it was established that significant differences existed between the mouthrinses, a Tukey test was carried out for a pair-wise comparison to determine which group differed from the others at a significance level of $p \leq 0.05$.

Table 6.6 represents the statistically significant differences between groups at a 95% confidence level for plaque index. It was found that all mouthrinses differed significantly from each other.

Dependent variable	Group	Group	Mean difference	Std. error	Sig.
PI	Listerine	Chlorhexidine	0.40133	0.02286	.000
	Listerine	Sterile water	-.60900	0.02286	.000
	Chx.	Listerine	-.40133	0.02286	.000
	Chx.	Sterile water	-1.01033	0.02286	.000
	Sterile water	Listerine	.60900	0.02286	.000
	Sterile water	Chlorhexidine	1.01033	0.02286	.000

Table 6.6: Multiple comparisons between groups (PI)

Table 6.7 represents the statistically significant differences between groups at a 95% confidence level for % PFS. It was found that all mouthrinses differed significantly from each other.

Dependent variable	Group	Group	Mean difference	Std. error	Sig.
% PFS	Listerine	Chlorhexidine	-29.783	.898	.000
	Listerine	Sterile water	11.800	.898	.000
	Chx.	Listerine	29.783	.898	.000
	Chx.	Sterile water	41.583	.898	.000
	Sterile water	Listerine	-11.800	.898	.000
	Sterile water	Chlorhexidine	-41.583	.898	.000

Table 6.7: Multiple comparisons between groups (% PFS)

Table 6.8 represents the statistically significant differences between groups at a 95% confidence level for % IPFS. It was found that all mouthrinses differed significantly from each other.

Dependent variable	Group	Group	Mean difference	Std. error	Sig.
% IPFS	Listerine	Chlorhexidine	-20.817	.847	.000
	Listerine	Sterile water	12.450	.847	.000
	Chx.	Listerine	20.817	.847	.000
	Chx.	Sterile water	33.267	.847	.000
	Sterile water	Listerine	-12.450	.847	.000
	Sterile water	Chlorhexidine	-33.267	.847	.000

Table 6.8: Multiple comparisons between groups (% IPFS)

CHAPTER 7

DISCUSSION

The present study showed the ability of the 4-day plaque regrowth study design to effectively demonstrate the differences in the anti-plaque properties of the different mouthrinses. Ramberg *et al* (1992) demonstrated that plaque formation completed in the first 4 days of growth remains the same in composition until day 14 of the plaque growth period. Based on these findings a 4-day period of plaque growth was acceptable for the study design.

Both active mouthrinses (Chlorhexidine and Listerine) reduced plaque formation compared to the control (saline), and this reduction was statistically significant. Listerine produced 25% of plaque-free surfaces, Chlorhexidine produced 54%, and sterile water, 13% plaque-free surfaces.

7.2.1- Chlorhexidine

The findings of this study demonstrate that Chlorhexidine is the most effective anti-plaque agent. This has been reported in several other studies (Overholser, 1990, Banting *et al,* 1989, Sergeto *et al,* 1986, Grossman *et al,* 1986, Lang *et al,* 1982, Loe *et al,* 1976, Flotra *et al,* 1972, Loe and Schiott, 1970). Chlorhexidine has a broad antimicrobial range (DePaola *et al,* 1996, Jenkins *et al*, 1994, Kubert *et al, 1993*).

That efficacy of Chlorhexidine can be attributed to the following factors:

Chlorhexidine is able to penetrate plaque and kill the bacteria inside the biofilm (Pan *et al,* 2000, Fine *et al,* 1996). Chlorhexidine has the affinity to bind to tissues and stay active for more than 12 hours (Netuschil *et al*, 1995, Weeks *et al*, 1988, Wolff, 1985, Roberts and Addy, 1981, Addy and Right, 1978, Turesky *et al,* 1977, Schiott, 1973).

But unfortunately because of some disadvantages it can't be used routinely or for a long time, of these disadvantages:

It was found in some studies that it causes taste alteration that can last up to 4 hours after rinsing with it (Ciancio, 2000).

Also in some studies tongue, teeth and restorations staining and supragingival calculus formation after the use of Chlorhexidine was noticed (Ciancio, 2000).

Also it should be used after 30 minutes of teeth brushing as recommended from the manufacturer because tooth paste ingredients will reduce its efficacy (Peridex, 2001).

7.2.2- Listerine

The results of this study concurred with previous findings that Listerine can reduce plaque formation (Gordon *et al,* 1985, Lamster *et al,* 1983, DePaola *et al,* 1989, Charles *et al,* 2000, Bauroth *et al,* 2003, Sharma *et al,* 2002, Sharma *et al*, 2004).

The ability of Listerine to reduce plaque can be attributed to the following factors:

Listerine has a broad antimicrobial range (DePaola *et al,* 1996, Jenkins *et al*, 1994, Kubert *et al,* 1993, Ross *et al,* 1989, Pitts *et al,* 1983).

Listerine is able to penetrate plaque and kill the bacteria inside the biofilm (Pan *et al,* 2000, Fine *et al,* 1996)

Listerine has the affinity to bind to tissues and stay active for several hours (Fine *et al,* 2001, Ross *et al,* 1989, DePaola, 1989).

In addition to all that the following advantages of Listerine make it able to be used as a routine adjunctive mouthrinse for long time:

Listerine doesn't stain the tongue, teeth and restorations (Charles *et al,* 2001, Overholser *et al,* 1990, DePaola *et al,* 1989, Gordon *et al,* 1985, Lamster *et al,* 1983).

Listerine doesn't change taste perception and it won't increase calculus formation (Charles *et al,* 2001, Overholser *et al*, 1990).

Listerine can be used at any time even immediately after brushing with out interfering with its efficacy (Santos, 2003).

The mean plaque scores during the trial period in the sample using Chlorhexidine are 0.5, and in the Listerine group 0.9. Both these scores are not an acceptable level of plaque control and, in fact, in the clinical situation it would be considered that

additional methods of oral hygiene need to be employed so as to prevent the initiation of disease.

One of the main recommendations for the use of mouthrinses was the inability for other methods of oral hygiene such as brushing and flossing to effectively eliminate plaque in the oral cavity. Manufacturers claim that in particular access to the interdental or interproximal surfaces was far better and effective if Chlorhexidine was used.

In this study, 42% of interproximal surfaces were rendered plaque-free in the group that used Chlorhexidine as a mouthrinse. Listerine was less effective and produced only 22% of interproximal surfaces plaque-free. On this basis, neither of these products can be used as a single method of oral hygiene and perhaps a further study needs to compare the efficacy of mechanical methods versus mouthrinses as the optimal method of oral hygiene.

Clearly, there is a role for the use of mouthrinses as an adjunct to mechanical methods of oral hygiene, particularly in patients who have problems with dexterity and in the elderly and infirm patients.

Another aspect of the use of mouthrinse as a means of oral hygiene, is that of the costs of the products. Locally (in South Africa), Chlorhexidine 0.2% can be dispensed at a very low cost to the patient and this is a further advantage of this product.

CHAPTER 8

CONCLUSIONS

This study evaluated the effects of essential oils mouthrinse (Listerine) on the 4-day interproximal plaque regrowth as compared to Chlorhexidine and sterile water.

The main conclusions are:

Chlorhexidine produced the highest percentage of plaque-free tooth surfaces (either of the entire tooth or the interproximal surfaces).

Listerine was a less effective anti-plaque agent than Chlorhexidine and sterile water was the least effective anti-plaque agent.

Although Listerine proved to be less effective than Chlorhexidine as an anti-plaque agent, it may still be used as an adjunctive anti-plaque mouthrinse with brushing and flossing.

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APPENDIX-I

EFFECTS OF MOUTH RINSE **A*** ON 4-DAY INTERPROXIMAL PLAQUE REGROWTH AS COMPARED TO MOUTHRINSES **B***and **C**

Researchers: Dr. A Jarrar

Prof. L Stephen

FACULTY OF DENTISTRY

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Volunteer instructions sheet

1- During both periods of the study after getting the base line scale and polish stop using any oral hygiene methods except for the allocated mouthrinse for the following 4 days.

2- Use the mouthrinse twice daily by rinsing with 20 ml in each time for 1 minute.

3- In the 2 weeks between the periods of the study practice your normal oral hygiene methods.

APPENDIX-II-A

Plaque index (Pl I) (Silness and Lőe, 1964) as described by Lőe, 1967 has the following scores:

Score **0** means: No plaque in the gingival area was found.

Score **1** means: A film of plaque adhering to the free gingival margin and the adjacent area of the tooth, and Plaque may only be recognized by running a probe across the tooth surface.

Score **2** means: Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and /or adjacent tooth surface, which can be seen by naked eye.

Score **3** means: Abundance of soft matter within the gingival pocket and /or on the gingival margin and adjacent tooth surface.

APPENDIX-II-B

1- Silness and Lőe examined only 4 surfaces on each tooth while the researcher examined 6 areas on each tooth.

2- Also the researcher used a disclosing agent in addition to the use of the probe as follows:

The volunteer used the disclosing agent first and from that the researcher got the following scores:

2=if the area was disclosed(less than 1/3 of the area cervico-incisally).

3=if the area was disclosed (more than 1/3 of the area cervico-incisally).

If the area wasn't disclosed with the agent, the probe was used to differentiate between scores **0**(no plaque on the probe) and **1**(there is plaque on the probe).

APPENDIX-III

Record Sheet

Volunteer code:

		8	8		
		7	7		
		6	6		
		5	5		
		4	4		
		3	3		
		2	2		
		1	1		
		1	1		
		2	2		
		3	3		
		4	4		
		5	5		
		6	6		
		7	7		
		8	8		

APPENDIX-IV

CONSENT FORM

EFFECTS OF MOUTH RINSE **A*** ON 4-DAY INTERPROXIMAL PLAQUE REGROWTH AS COMPARED TO MOUTHRINSES **B*** AND **C***

Researchers: **Dr. A Jarrar**

Prof. L Stephen

FACULTY OF DENTISTRY

UNIVERSITY OF WESTERN CAPE

The purpose of this study is to find out the effects of mouth rinse A on 4-day Interproximal plaque regrowth. The researcher will compare the effect of that mouth rinse with mouthrinses B and C.

People who will participate in the study will have a professional cleaning of their teeth at the beginning of each of the three periods of the study, then they will be asked to use the allocated mouthrinse for the following 4-days as the only oral hygiene method (it is known that 4 days are not enough period to start periodontal disease, because it needs at least 21 days to be established) (Theilade *et al,* 1966, Lőe *et al,* 1965) and according to the instructions that will be given orally and on written paper. On day 5 volunteers will be examined to see the amount of plaque. The second and third periods will be the same except for using the other mouthrinses.

The results of the study will help to determine if those commercially available mouthrinses are effective really in Interproximal areas or not.

Every participant will get free professional teeth cleaning and oral examination and advice and referral for any treatment if needed.

Participation in the study will be on voluntary basis. Participants will be expected not to have any harmful effects. If a participant wants to withdraw from the study at any stage, he/she will be free to do so.

The information obtained in the study will be treated with utmost confidentiality and the names of the participants will not be divulged for any other purpose.

For any further information, please contact the undersigned:

Dr AHMED JARRAR

Phone number: 082-8351701

E-mail: ajarrar@uwc.ac.za

Consent

I have read the information, asked questions, and received answers concerning areas that were unclear and willingly agree to participate in this study. My participation is completely voluntary. I may withdraw at any time without any treatment being held from me. I will not have waived any of my legal rights by signing this consent form. Upon signing this form, I will receive a copy of the entire consent.

……	……	…….
Participant Name	Signature	Date
……	……...	……..
Witness Name	Signature	Date