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**THE EFFECTIVENESS OF USING A PERIODONTAL
ENDOSCOPE AS AN ADJUNCT TO NON-SURGICAL
PERIODONTAL THERAPY: CLINICAL, RADIOGRAPHIC
AND MICROBIOLOGICAL RESULTS**

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BOH MPH**

A thesis submitted fulfilling all of the requirements for the degree of
Doctor of Philosophy

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Melbourne Dental School
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DECLARATION

THIS IS TO CERTIFY THAT:

1. THIS THESIS IS MY ORIGINAL WORK AND THAT I HAVE MADE ACKNOWLEDGMENTS IN THE TEXT OF ALL OTHER MATERIALS USED IN THIS THESIS.
2. THE THESIS IS LESS THAN 100 000 WORDS IN LENGTH, EXCLUDING LIST OF TABLES, FIGURES, BIBLIOGRAPHY AS APPROVED BY THE RESEARCH HIGHER DEGREES COMMITTEE, UNIVERSITY OF MELBOURNE.

Meloshini Naicker

Date

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ABBREVIATIONS

AAP	American Academy of Periodontology
AL	Attachment loss
AR	Adam Rosenberg
BANA	N-benzoyl-DL-arginine-2-naphthylamide
BCFIA	Bacterial concentration fluorescence immunoassay
BOP	Bleeding on probing
BI	Bleeding index
CADIA	Computer-assisted densitometric image analysis
CAL	Clinical attachment level
CBCT	Cone beam computerized tomography
CDC	The US Centre for Disease Prevention
CEJ	Cemento-enamel junction
C	Control group-SRD only
CS	Coronal scaling
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
rDNA	Recombinant deoxyribonucleic acid
EFP	European Federation of Periodontology
ELISA	Enzyme-linked immunosorbent assay
EBI	Endoscopic biofilm index
EII	Endoscopic inflammation index
ECI	Endoscopic calculus index
EPA	Eicosapentaenoic acid
Er: YAG	Erbium: yttrium-aluminium-garnet
FO	Flap with osseous resection surgery
F/up	Follow up
GI	Gingival index
HbA1c	Glycated haemoglobin
HEAG	Health Sciences Human Ethics Sub-Committee
HIV	Human immunodeficiency virus
HMT	Host modulation therapy
HOMIM	Human Oral Microbe Identification Microarray

NAOHS	National survey of adult oral health
NH ₃	Ammonia
H ₂ S	Hydrogen Sulphide
IL-6	interleukin- 6
IL-8	interleukin- 8
LCD	Liquid crystal display
LCn3PUFA	Long-chain omega-3 polyunsaturated fatty acids
MN	Meloshini Naicker
MW	Modified widman flap surgery
OHI	Oral hygiene instruction
NA	Not available
Nd:YAG	Neodymium: yttrium-aluminium-garnet
No	Number
NRMH	No relevant medical history
PAL	Probing attachment level
PCR	Polymerase chain reaction
qPCR	Quantitative real-time polymerase chain reaction
PI	Plaque index
PPD	Probing pocket depth
RBL	Radiographic bone level
RNA	Ribonucleic acid
RCT	Randomised controlled trial
ROQT	RNA-oligonucleotide quantification technique
RP	Root planing
SBI	Subgingival biofilm index
SCI	Subgingival calculus index
SF	Sue Finch
SPT	Supportive periodontal maintenance therapy
SRD	Scaling and root debridement
SRP	Scaling and root planing
T	Test group-SRD with perioscope
3-D	Three dimensional
16srRNA	16S ribosomal ribonucleic acid
VSC	Volatile sulphide compounds

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ABSTRACT

Non-surgical periodontal therapy has been one of the main treatment approaches for managing patients with periodontal disease for decades. The aim of this treatment is to remove bacteria and subgingival deposits, create a “clinically healthy” environment and improve microbial levels to levels that is compatible with health. Conventional non-surgical debridement includes both hand and powered instruments with the ideal end point being a smooth root surface. Periodontal endoscopy was developed in the late 1990s and features miniaturized digital video technology allowing the operator to directly visualise the subgingival environment and at the same time remove any calculus or debris from the root surface with the use of hand or powered instruments. The benefits of direct visualisation technology in improving clinical and inflammatory outcomes were demonstrated in retrospective and prospective studies as well as randomised controlled studies utilizing either split-mouth design or parallel design. However, there was a need to investigate if endoscope scaling and root debridement (SRD) is more effective in reducing levels and numbers of periodontal pathogens as compared to conventional nonsurgical treatment. Also, if bacterial counts decrease, can they be maintained or reduced even further with strict three monthly supportive periodontal maintenance therapy (SPT). Osseous changes can occur after nonsurgical periodontal therapy. However, it is often difficult to determine if changes have occurred due to limitations with conventional radiography. This study included the use of standardized radiographs using customized positioning stents and paralleling x-ray holding devices. Digital radiography and digital software were used to determine areas of osseous change.

Aims

1. To compare endoscope-assisted SRD (SRD-with endoscope) to traditional SRD (SRD-only) in reducing clinical parameters over a 12-month period.
2. To assess if endoscope assisted SRD shows more evidence of radiographic changes after the 12-month period compared to conventional SRD.

3. To compare bacterial counts of 11 species including *Aggregatibacter (Actinobacillus) actinomycetemcomitans* (*A. actinomycetemcomitans*); the red complex (*Porphyromonas gingivalis* (*P. gingivalis*); *Tannerella forsythia* (*T. forsythia*) and *Treponema denticola* (*T. denticola*)); the orange complex (*Prevotella intermedia* (*P. intermedia*); *Peptostreptococcus micros* (*P. micros*) ; *Fusobacterium nucleatum/periodonticum* (*F. nucleatum*)); the orange-associated complex (*Campylobacter rectus* (*C. rectus*); *Eubacterium nodatum* (*E. nodatum*)); the green complex (*Eikenella corrodens* (*E. corrodens*)) and *Capnocytophaga* species (*Capnocytophaga* sp.) (*C. sputigena*; *C. gingivalis*; *C. ochracea*)) in both groups over a 12-month period.
4. To determine the need for surgical intervention in both test and control groups after the 12-month treatment.

Materials and Methods

The study included 38 participants diagnosed with chronic moderate to advanced periodontitis. Nineteen participants were included in the test group (SRD-with perioscope) and 19 in the control group (SRD-only) by consecutive allocation. Clinical examination included probing pocket depths (PPD), probing attachment levels (PAL), gingival recession, assessment of furcation, mobility, bleeding on probing (BOP) and plaque scores (PI). The measurements were recorded at baseline, three and twelve months and differences in means between groups were calculated for all clinical parameters.

The Hain Lifescience Micro-IDent test was utilized for the microbial analyses. Five sterile paper points were inserted into five of the deepest pockets, placed into a sterile test tube and sent in a water-resistant bag to Nehren, Germany for analysis. Eleven putative pathogens namely *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia*, *T. denticola*, *P. micro*, *F. nucleatum*, *C. rectus*, *E. nodatum*, *E. corrodens* and *Capnocytophaga* sp., were analysed at each time point and compared between the control (SRD-only) and test group (SRD-with perioscope). Analysis of pathogens in their respective complexes namely the red, orange, orange-associated and green

complexes were also included. Standardised radiographs were taken at sites with the deepest pockets and in sites displaying angular/vertical bone loss using positioning stents at the baseline and 12-months. The Digimizer Image Analysis software (©2005-2018 MedCalc Software bvba, Belgium) was used to measure from the cemento-enamel junction (CEJ) to the alveolar bone crest and changes were tracked in millimetres between radiographs taken pre-SRD and at twelve months. Linear measurements were utilized to determine mean radiographic bone levels (RBL).

Results

There were no significant differences between groups with regards to age, gender, medical history and disease severity. Both groups showed significant improvements in all clinical parameters after therapy ($p < 0.05$). At three months, no statistically significant differences could be found between groups with mean PPD, mean PAL, BOP and PI. However, for PPDs 7-9 mm the test group had a significantly lower percentage as compared to the control group. At twelve months, the mean PPD was found to be significantly lower in the test group (2.70 ± 0.2 mm) as compared to the control group (2.96 ± 0.4 mm) ($p < 0.05$). In addition, the test group also had lower BOP ($4.3 \pm 3.2\%$) percentage as compared to the control group ($11.95 \pm 7.1\%$). PI percentage ($25.61 \pm 3.9\%$) was also reported to be lower in this group as compared to the control group ($30.11 \pm 6.3\%$). The test group had less change in gingival recession (-0.13 ± 0.2 mm) as compared to the control group (-0.5 ± 0.6 mm) from baseline to twelve months ($p < 0.05$).

There were no statistically significant differences found between the test and control groups with regards to reduction of periodontal pathogens and their complexes ($p > 0.05$). Both SRD-only and SRD-with perioscope resulted in decreases in numbers of periodontal pathogens including, *P. gingivalis*, *T. forsythia*, *T. denticola*, *P. intermedia*, *P. micro*, *E. nodatum* and *E. corrodens* post-therapy and at twelve months with numbers remaining below pre-treatment levels. However, *A. actinomycetemcomitans*, *C. rectus*, and *Capnocytophaga* sp. decreased post-therapy in both groups but increased at twelve months. *F. nucleatum* increased in both groups post-SRD and then were reported at lower levels at twelve months. Both the red and

orange complex had lower numbers in the test group at twelve months, with the control group having lower numbers of the green and orange-associated complexes.

The mean change in RBL was significantly higher in the test group (0.69 ± 0.3 mm) as compared to the control group (0.49 ± 0.2 mm) ($p < 0.05$). This positive change in mean RBL is indicative of more radiographic bone gain in the test group. There were no differences reported between groups with regards to mean change in RBL for single-rooted teeth ($p > 0.05$). However, for multi-rooted teeth more radiographic bone gain was observed in the test group (0.83 ± 0.5 mm) with a higher mean change in RBL as compared to the control group (0.46 ± 0.4 mm) ($p < 0.05$). The test group had a higher frequency of RBL between 0.5 mm and 1.0 mm and 1.0 mm to 1.5 mm as compared to the control group, inferring that the test group had more sites with radiographic bone gain in this range as compared to the control group.

Conclusions

Both non-surgical treatment methods used resulted in positive outcomes in clinical, microbiological and radiographic parameters. The adjunctive use of the perioscope significantly improved PPDs 7-9 mm at three and twelve months. The mean PPD at twelve months was significantly lower in the test group as compared to the control group. Less change in gingival recession was observed using the endoscope. The test group had significantly lower BOP% and PI% at twelve months. No significant differences between groups were observed with analyses of the eleven pathogens and complexes of bacteria. The significantly higher mean RBL observed in the test group as compared to the control group is suggestive of more radiographic bone gain in this group. This outcome was also observed for multi-rooted teeth in the test group.

CHAPTER 1

BACKGROUND

1.1 Periodontal Disease

1.1.1 Defining Periodontal Disease

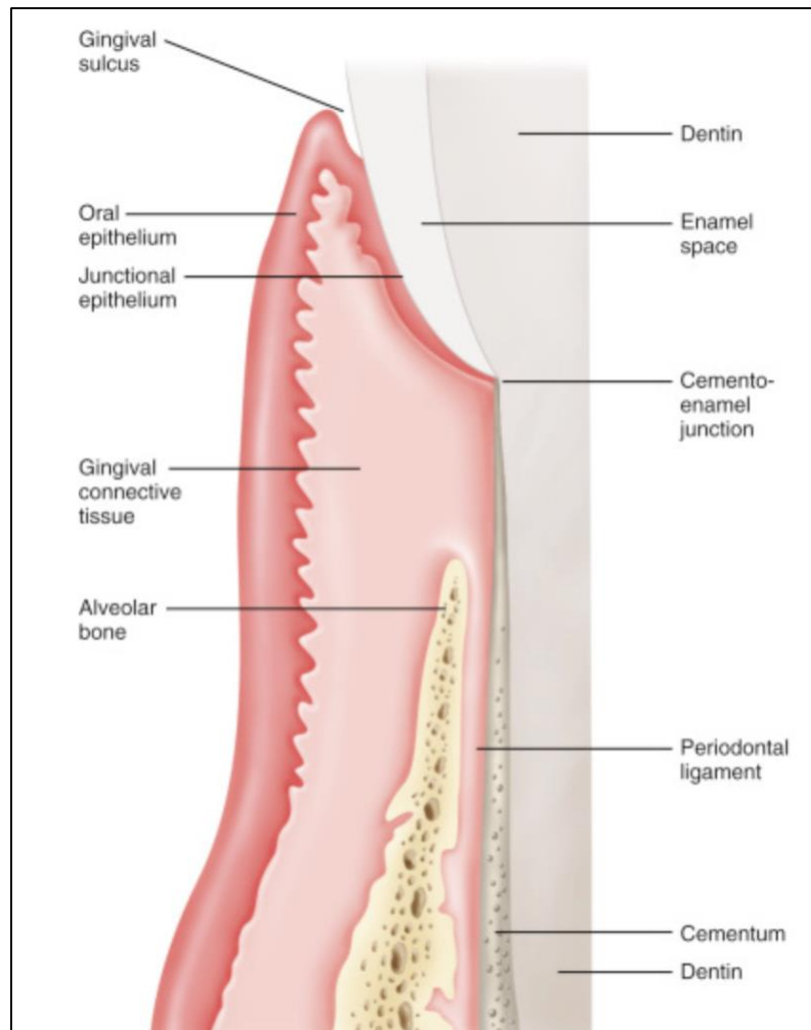
Periodontal disease refers to both gingivitis and periodontitis. Plaque induced gingivitis, the most common form of gingivitis, is characterized by an inflammatory condition confined to the gingiva, without attachment loss (AL), which normally can be reversed with good oral hygiene. This type of gingivitis may persist without progression into periodontitis (Wilson *et al.*, 1996).

Periodontitis is considered to be polymicrobial infection, involving a complex interaction between microorganisms in the biofilm, the local environment and the host immune response. It is a slowly progressing disease which is typically asymptomatic and is characterized by inflammation of the supporting tissues of the teeth, bone and soft tissue with loss of connective tissue, destruction of collagen fibres and apical migration of the junctional epithelium. This destruction of the supporting structures of teeth can lead to eventual tooth loss (Kinane, 2001; Sanz *et al.*, 2011; Teles *et al.*, 2011).

1.1.2 Anatomy of the Periodontium

The periodontium consists of the gingiva, periodontal ligament, root cementum and alveolar bone. The gingiva has two parts namely the free gingiva and the attached gingiva. The gingival sulcus is formed between the tooth and the sulcular epithelium. In healthy gingiva, the gingiva is in close contact with the tooth surface. The periodontal ligament is a connective tissue bundle made up of fibre bundles and cells with a width of approximately 0.25 mm (range 0.2-0.4 mm). It provides support during mastication and proprioception (Lindhe *et al.*, 2008).(Figure 1.1)

Figure 1. 1 Cross section of the periodontium



Sourced from *Periodontics: medicine, surgery and implants*, St. Louis, 2004, Mosby (Rose *et al.*, 2004).

1.1.3 Pathogenesis of Periodontal disease

Page and Schroeder in 1976 published a landmark review paper of concepts surrounding the pathogenesis of periodontal disease. These concepts have helped us understand that although bacteria are a requirement for periodontal disease it is also the host response and the host-microbial balance that can result in stages of disease progression and remission.

1.1.3.1 Gingival Health

The healthy periodontium has no signs of clinical inflammation, with the gingival tissues appearing coral-pink or pigmented in colour, with or without stippling, has a firm consistency with the marginal gingiva following a scalloped line and lying flat to the tooth. Very few inflammatory cells, mainly polymorphonuclear lymphocytes, are found within the connective tissue and junctional epithelium and the alveolar bone is 2-3 mm apical to the junctional epithelium (Perry *et al.* 1996, Wilson *et al.* 1996).

1.1.3.2 Initial Lesion

Within hours of plaque accumulation, vascular changes typical of acute inflammatory responses are observed, however these are not clinically visible. These following changes are seen:

- Blood vessels adjacent to the junctional epithelium become enlarged and more permeable
- Migration of polymorphonuclear leukocytes into the gingival sulcus and junctional epithelium
- Lymphocytes accumulate adjacent to the junctional epithelium with only few plasma cells present
- Morphologic changes in the fibroblasts
- Proliferation of epithelial cells and loss of collagen surrounding the blood vessels

1.1.3.3 Early Lesion

The early lesion occurs 7 days of plaque accumulation. The early lesion is characterised by:

- Increase in vascular changes
- Fluid exudation and migration of polymorphonuclear leukocytes
- Lymphocytes and macrophages adjacent to the junctional epithelium
- Cytopathic changes are observed in the fibroblasts
- Further loss of collagen adjacent to the junctional epithelium is evident
- Proliferation of the cells of the junctional epithelium

- Clinical signs of oedema and erythema visible.

1.1.3.4 Established Lesion

The early lesion progresses into an established lesion about 2 to 3 weeks of plaque accumulation. The established lesion is characterised by:

- Clinically, more redness and gingival swelling (false pocket)
- Plasma cells and lymphocytes predominate at the periphery of the lesion, but without bone loss
- Accumulation of both macrophages and lymphocytes in the lamina propria of the gingival pocket
- Continuous loss of collagen fibres and fibrous connective tissue.
- Proliferation, apical migration of the junctional epithelium and lateral extension of the junctional epithelium

1.1.3.5 Advanced Lesion

The same features as described for the established lesion is seen with extension of the lesion into the alveolar bone and periodontal ligament with bone loss.

- Changes from T-cell to B-cell predominance marks the change from gingivitis to periodontitis
- Continuous loss of collagen fibres and matrix into the pocket epithelium and in the periodontal ligament space.
- Periodontal pocket formation and apical migration of the junctional epithelium.

(Kinane, 2001; Page *et al.*, 1997b)

1.1.4 Classification of Periodontal Disease

1.1.4.1 The 1999 Periodontal Disease Classification

Over the years, there have been many amendments to the classification of periodontal disease. In 1996, the World Workshop of Periodontics recognized a need to revise the classification as the 1986 classification did not include gingival diseases, nor did it take into account systemic factors, medications and factors such as malnutrition. The term “adult periodontitis” created debate as clinicians found that both adolescents and adults can present with these signs and symptoms, thus “adult periodontitis” was replaced with “chronic periodontitis”. Rates of progression of disease, rather than age were considered when re-classifying periodontal disease; hence, early onset periodontal disease was renamed using the term “aggressive periodontitis”. Refractory periodontal disease, which was initially classified as a separate entity, was now applied to all forms of the disease. Other changes included the changing of “necrotizing ulcerative periodontitis” with “necrotizing periodontal diseases” and the inclusion of “periodontitis associated with endodontic lesions” and “developmental or acquired deformities and conditions” (Armitage, 1999).

The classification of periodontal disease listed below is based on the 1999 International Workshop and is as follows:

- Gingival Diseases
- Chronic Periodontitis
- Aggressive Periodontitis
- Periodontitis as a manifestation of systemic diseases
- Necrotizing Periodontal Diseases
- Abscesses of the periodontium
- Periodontitis Associated with Endodontic Lesions
- Developmental or acquired deformities and conditions (Armitage, 2002)

The 1999 classification has been used for the last 19 years and with new information emerging from population, prospective and science studies, there was a need to update the classification to include this new evidence.

1.1.4.2 2017 World Workshop Classification of Periodontal and Peri-Implant Diseases and Conditions

In 2015, the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP) appointed a task force to address specific issues with the current classification used. Specific areas of concern including clinical attachment level, chronic versus aggressive periodontitis and localized versus generalized periodontitis were addressed, as well as the need for the inclusion of peri-implant disease and conditions.

The report of the World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions was announced at the EuroPerio9 congress in 2018 in Amsterdam. This classification takes into consideration new information related to the epidemiology, etiology and pathogenesis of periodontitis and aims to assist the clinician with diagnosis and well as management of patients (Caton *et al.*, 2018).

“Gingival diseases” was replaced by “Periodontal Health and Gingival Diseases and Conditions” which includes “Periodontal Health and Gingival Health”; “Gingivitis: Dental Biofilm-Induced” and “Gingival Diseases: Non-Dental Biofilm Induced”.

Unresolved issues such as the difference between presence of gingival inflammation at one or more sites and the definition of gingivitis were emphasized. Consensus was reached that BOP should be used as the key marker to set levels for gingivitis. Consideration was given to periodontal health and gingival inflammation in a reduced periodontium after completion of periodontal treatment based on BOP and PPDs. The reasoning behind this was to highlight the need for continual SPT and monitoring of periodontal patients who have been treated and had a successful outcome. Based on the amount of inflammation and improvement of health of the reduced periodontium,

periodontal health can be classified as “Periodontal disease stability” or “Periodontal disease remission/control” (Lang *et al.*, 2018). Non-plaque induced gingival diseases and conditions were re-organised into “gingival-dental biofilm induced diseases” for example “drug-influenced gingival enlargement” and “non-dental biofilm induced” such as genetic /developmental disorders (Caton *et al.*, 2018).

It was agreed that three forms of Periodontitis exist. This includes “Necrotizing Periodontal Diseases”; “Periodontitis as a Manifestation of Systemic Diseases” and forms of disease previously referred to as “chronic” and “aggressive” now grouped under “Periodontitis”. “Periodontitis as a Manifestation of Systemic Diseases” include rare disorders such as “Papillon Lefèvre Syndrome” that cause early presentation of severe periodontitis (Papapanou *et al.*, 2017).

1.1.4.2.1 Staging and Grading

“Periodontitis” was further characterized depending on two factors, namely staging and grading. Staging is determined by the extent, severity and complexity of disease. Factors such as clinical attachment loss, amount and percentage of bone loss, PPDs, presence and magnitude of angular bone defects, furcation involvement, mobility and loss of teeth due to periodontitis are considered (Caton *et al.*, 2018).

Staging is as follows:

- Stage I: Initial Periodontitis (early stages of AL)
- Stage II: Moderate Periodontitis (established periodontitis)
- Stage III: Severe Periodontitis significant damage to the periodontium with potential for additional tooth loss
- Stage IV: Advanced Stage of Periodontitis with potential for loss of dentition.

(Figure1.2)

Figure 1. 2 Periodontitis staging

Periodontitis stage		Stage I	Stage II	Stage III	Stage IV
Severity	Interdental CAL at site of greatest loss	1 to 2 mm	3 to 4 mm	≥5 mm	≥5 mm
	Radiographic bone loss	Coronal third (<15%)	Coronal third (15% to 33%)	Extending to middle or apical third of the root	Extending to middle or apical third of the root
	Tooth loss	No tooth loss due to periodontitis		Tooth loss due to periodontitis of ≤4 teeth	Tooth loss due to periodontitis of ≥5 teeth
Complexity	Local	Maximum probing depth ≤4 mm	Maximum probing depth ≤5 mm	In addition to stage II complexity: Probing depth ≥6 mm	In addition to stage III complexity: Need for complex rehabilitation due to:
		Mostly horizontal bone loss	Mostly horizontal bone loss	Vertical bone loss ≥3 mm Furcation involvement Class II or III Moderate ridge defect	Masticatory dysfunction Secondary occlusal trauma (tooth mobility degree ≥2) Severe ridge defect Bite collapse, drifting, flaring Less than 20 remaining teeth (10 opposing pairs)
Extent and distribution	Add to stage as descriptor	For each stage, describe extent as localized (<30% of teeth involved), generalized, or molar/incisor pattern			

Sourced from (Tonetti *et al.*, 2018)

The extent and distribution are also considered, whether it is localised, generalised and molar-incisor distribution (Caton *et al.*, 2018; Tonetti *et al.*, 2018).

Grading, on the other hand takes into consideration rate of progression, risks associated with it and anticipated treatment response. Factors related to periodontal progression but also factors such as smoking, diabetes, general health status are considered when grading. Grading allows treatment plans to include individual patient factors.

There are three risk levels namely:

Grade A: low risk, slow rate of progression

Grade B: moderate risk, moderate rate of progression

Grade C: high risk for rapid rate for progression.

(Caton *et al.*, 2018; Tonetti *et al.*, 2018)

Figure 1.3

Figure 1. 3 Periodontitis grading

Periodontitis grade			Grade A: Slow rate of progression	Grade B: Moderate rate of progression	Grade C: Rapid rate of progression
Primary criteria	Direct evidence of progression	Longitudinal data (radiographic bone loss or CAL)	Evidence of no loss over 5 years	<2 mm over 5 years	≥2 mm over 5 years
	Indirect evidence of progression	% bone loss/age	<0.25	0.25 to 1.0	>1.0
		Case phenotype	Heavy biofilm deposits with low levels of destruction	Destruction commensurate with biofilm deposits	Destruction exceeds expectation given biofilm deposits; specific clinical patterns suggestive of periods of rapid progression and/or early onset disease (e.g., molar/incisor pattern; lack of expected response to standard bacterial control therapies)
Grade modifiers	Risk factors	Smoking	Non-smoker	Smoker <10 cigarettes/day	Smoker ≥10 cigarettes/day
		Diabetes	Normoglycemic / no diagnosis of diabetes	HbA1c <7.0% in patients with diabetes	HbA1c ≥7.0% in patients with diabetes
Risk of systemic impact of periodontitis ^a	Inflammatory burden	High sensitivity CRP (hsCRP)	<1 mg/L	1 to 3 mg/L	>3 mg/L
Biomarkers	Indicators of CAL/bone loss	Saliva, gingival crevicular fluid, serum	?	?	?

Sourced from (Tonetti *et al.*, 2018)

1.1.4.2.2 Other Inclusions in The Classification

Other conditions affecting the periodontium included in the classification are:

- Systemic disease or conditions affecting the periodontal supporting tissues
- Periodontal Abscesses and Endodontic Periodontal Lesions
- Mucogingival Deformities and Conditions
- Traumatic Occlusal Forces
- Tooth and Prosthesis Related Factors

(Caton *et al.*, 2018; Papantonopoulos, 2004).

Neoplastic diseases may adversely affect the periodontium and not be related to “dental plaque induced periodontitis” and these can be categorised as “Systemic

Diseases or Conditions affecting the Periodontal Supporting Tissues”. Systemic diseases such as diabetes mellitus are included in the staging and grading process.

Treatment of gingival recession considering interproximal clinical attachment loss and the evaluation of exposed root and CEJ are included. The phrase “periodontal biotype” was substituted by “periodontal phenotype”. The term “biologic width” was changed to “supracrestal attached tissues” in prosthesis and tooth-related factors. Traumatic occlusal forces replaced excessive occlusal force (Caton *et al.*, 2018; Papapanou *et al.*, 2017).

Peri-implant diseases and conditions are incorporated in the 2017 classification. Peri-implant health, peri-implant mucositis and peri-implantitis as well as hard and soft tissue implant site deficiencies were recognized (Caton *et al.*, 2018).

1.1.4.2.3 Definition Used in This Thesis

For the current study, the 1999 classification was used as the study began before the 2017 classification was released. Patients diagnosed with chronic periodontitis were considered for inclusion. Chronic periodontitis is usually characterized by a slowly progressive disease which starts as plaque-induced gingivitis (Armitage, 1999; Lindhe *et al.*, 2008). It is the most common type of periodontal disease found in humans. The clinical features include changes in colour, texture and consistency of marginal gingiva, BOP, increasing pockets depths or periodontal pocketing, AL, loss of alveolar bone, furcation involvement and mobility of teeth (Lindhe *et al.*, 2008). Chronic periodontitis can follow a slow progression or can have brief episodes of rapid progression followed by periods of remission (Mlachkova *et al.*, 2014).

1.2 Prevalence of Periodontal Disease in Australia

Periodontal disease is considered highly prevalent, yet a hidden chronic inflammatory disease as it is relatively asymptomatic and slow progressing. Patients are usually unaware of this disease until the condition has progressed enough to become

symptomatic. The US Centre for Disease Prevention (CDC) and AAP defined moderate periodontitis “as the presence of either two sites between adjacent teeth where the gum has lost its attachment to the tooth for 4 mm or more, or at least two such sites that have pockets of 5 mm or more (ARCPOH, 2009). Using this CDC/AAP definition, the prevalence of periodontitis in Australia were reported to be 22.9%.

The National Survey of Adult Oral Health (2004-2006) (NAOHS) conducted in Australia included 14,123 patients. The study revealed that the prevalence of moderate or severe periodontal disease was higher in older age groups and ranged from 2.7% (ages 15–24) to 53.4% at age 65 and over (Chrisopoulos *et al.*, 2013). Almost one in five people (19.8%) had periodontal pocketing greater than 4 mm with the prevalence of periodontal pocketing reported to be 1.4 times higher in males as compared to females (22.8% versus 16.7%) in all age groups. It was also found that 42.5% of the Australian population had clinical attachment loss of 4 mm or more (Slade *et al.*, 2007). The occurrence of periodontal disease was also influenced by location, with people in the major cities having the lowest occurrence of periodontal disease (22.1%) as compared to those in remote areas (36.3%). Factors such as whether people had health insurance or not and level of household income also influenced occurrence of periodontal disease (Chrisopoulos *et al.*, 2013). The NAOHS also reported that the estimated prevalence of periodontal disease amongst Indigenous Australians was higher (29%) when compared to non-Indigenous Australians (23%) (Chrisopoulos *et al.*, 2013). This was further evidenced by a survey amongst 312 Indigenous Australians, where 87.5% of this population were confirmed periodontitis cases, which was 3.5 times higher than that estimated at the national oral health survey (Kapellas *et al.*, 2014).

1.3 Risk Factors for Periodontitis

Research into the pathogenesis of periodontitis has been linked to specific inherited, behavioural and environmental factors contributing to the susceptibility and progression of this disease. Periodontal disease is no longer considered as a simple bacterial infection. Factors such as subgingival microbiota, the host immune and

inflammatory response as well as environmental factors need to be taken into consideration (Lang *et al.*, 2018).

Smoking, stress, drugs, nutrition, diabetes and many other systemic conditions are considered as risk factors. Factors such as genetics, age, gender and socio-economic status, have also been linked with periodontal diseases. However, these are considered risk indicators or determinants (Genco *et al.*, 2013; Irwin *et al.*, 2007; Page *et al.*, 1997a). Variability to susceptibility to periodontal disease is not fully understood. Studies have shown that both the susceptibility and severity of disease is not the same in all patients. A retrospective study of 600 patients with moderate to advanced periodontal disease, reported that only 16% of patients accounted for 58% of the teeth that were lost to periodontal disease. Teeth with the most gingival inflammation did not lose bone support or teeth over the years (Hirschfeld *et al.*, 1978). Associated factors may increase the risk of initiation or progression of disease by disturbing the balance between periodontal microbes and the host, resulting in periodontal destruction (Salvi *et al.*, 1997).

However, risk factors, risk indicators and determinants are important to consider when managing and treatment planning for periodontal diseases. Both modifiable risk factors such as smoking and diabetes as well as non-modifiable factors such as genetics can affect treatment outcomes (Darby, 2009). The grading included in the 2017 periodontal classification takes into consideration risk factors such as smoking and diabetes. These factors affect the rate of progression of the disease as well as increase the risk of progression from one stage to another. Obesity, genetic factors and nutrition are considered in the 2017 classification. However, a flexible approach is recommended as new evidence emerges (Tonetti *et al.*, 2018).

1.3.1 Smoking and Periodontal Disease

Smoking has been acknowledged as a significant risk factor for destructive periodontal disease. Its relationship with periodontal disease has been researched extensively and is still an ongoing topic of research. Smoking has been linked to the initiation, extent and severity of periodontal disease (Bergstrom, 2003).

The cross sectional study by Grossi *et al.*, (1994), reported a dose dependant relationship between increasing consumption of cigarettes and risk of periodontitis as measured by AL (Grossi *et al.*, 1994). This was further evidenced by Bergstrom *et al.*, (2000). This ten year prospective study found an increase in the number of diseased sites (PPD $\geq$ 4 mm) as well as periodontal bone destruction in smokers as compared to non-smokers (Bergstrom *et al.*, 2000). Stoltenberg *et al.*, (1993) reported that not only did smokers have greater mean pocket depths (3.12 mm) compared to non-smokers (2.94 mm), but the odds of smokers having pocket depths greater than 3.5 mm were 5.3 times greater than non-smokers (Stoltenberg *et al.*, 1993). Nunn (2003) concurred with smokers being 2.7 times more likely to have moderate to advanced periodontal disease as compared to non-smokers (Nunn, 2003).

The systematic review by Bergstrom (2006), which included cross-sectional, cohort and case studies, demonstrated consistently that periodontal condition in smokers is significantly inferior to non-smokers. One hundred percent of the 70 cross sectional studies demonstrated a definite association between smoking and an impaired periodontium, with smokers exhibiting greater periodontal morbidity than non-smokers. The case control studies analysed in this review found that smokers displayed more severe signs and symptoms of periodontal disease when compared to non-smokers. Ninety-five percent of the 21 cohort studies showed that an association exists between the onset and progression of periodontal disease and smoking. However, cohort studies can underrate the true effects of smoking because of attrition rates (Bergstrom, 2006).

The study by Tomar and Asma in 2000 examined the association between smoking and periodontitis and estimated the proportion of periodontitis in the adult population in the United States related to cigarette smoking. After consideration was given for age, gender, race, ethnicity, education and income: poverty ratio, it was reported that current smokers were four times more likely to have periodontitis as compared to non-smokers. A dose-dependent relationship was observed between number of cigarettes smoked per day and the odds of having periodontitis. The odds of having periodontitis reduced with the number of years quitting in former smokers. This study found that 41.9% (6.4 million cases) of periodontitis cases in the US adult population was

correlated to current cigarette smoking, while 10.9% (1.7 million cases) were related to former smoking. The percentage of periodontitis attributable to current smokers were reported as 74.8%. This study confirmed that smoking is a major risk factor and a large proportion can be prevented through prevention and smoking cessation programmes (Tomar *et al.*, 2000).

Smoking cessation has shown to have beneficial effects in reducing pocket depths following non-surgical periodontal therapy (Rosa *et al.*, 2014; Sherwin *et al.*, 2013). A 24-month prospective study by Rosa *et al.*, (2014) assessed the effect of smoking cessation on non-surgical periodontal therapy in adults with chronic periodontitis. The eighteen participants that quit smoking showed a significantly higher mean clinical attachment gain in diseased areas and greater reduction in the proportion of sites with clinical attachment levels ≥ 3 mm when compared to non-quitters (n=32). In addition, quitters showed a significantly higher mean probing depth reduction as compared to non-quitters ($p \leq 0.05$) (Rosa *et al.*, 2014). This concurs with the 10-year prospective study in 2000 by Bergstrom *et al.*, that revealed that the periodontal condition of non-smokers and former smokers remained stable during the 10 year period suggesting that smoking cessation is favourable toward periodontal health (Bergstrom *et al.*, 2000).

The adverse effects smoking has on periodontal treatment outcomes have been demonstrated in many studies (Borojevic, 2012; Sherwin *et al.*, 2013). In 1994, Ah *et al.*, evaluated the effect of smoking on the clinical response to both surgical and non-surgical periodontal therapy. Treatment modalities included coronal scaling (CS), scaling and root planning (SRP), modified Widman's flap (MW), and a flap with osseous resection (FOP). Following active therapy, patients were followed for 6 years for SPT. The results demonstrated that in smokers, the response to periodontal treatment was less favourable as compared to non-smokers. Smokers showed less improvement in pocket depths and attachment level gains and greater horizontal AL at furcation sites (Ah *et al.*, 1994).

Smoking affects the vasculature and has immunologic reactions (Sherwin *et al.*, 2013). The study by Peruzzo *et al.*, (2016) involved an experimental gingivitis model that assessed the effect of smoking on parameters including clinical, microbiological and

immunological response. It was found that gingival inflammation developed in smokers and non-smokers; however, there was less bleeding in smokers when compared to non-smokers. This result is keeping with what is known on the effects of smoking on the inflammatory response, whereby nicotine smoke by-products lead to vasoconstriction, reduction of blood flow and oedema which masks the clinical signs of inflammation. This study also found higher proportions of periodontal pathogens in smokers than non-smokers. Mean total bacterial counts were higher in smokers. The red and orange complex bacteria were found in higher proportions while the beneficial bacterial species, such as *Actinomyces* species, and purple and yellow complex bacteria were detected at lower proportions in this group. Smokers also demonstrated a distinct host-response pattern during the course of experimental gingivitis (Peruzzo *et al.*, 2016). Specific clusters of periodontal pathogens were also found in smokers in the study by van Winkelhoff *et al.*, (2001) namely *Bacteriodes forsythus* (*B. forsythus*), *P. micros*, *F. nucleatum* and *C. rectus*, suggesting that the composition of subgingival pathogens can be influenced by smoking (van Winkelhoff *et al.*, 2001).

1.3.2 Diabetes and Periodontal Disease

Diabetes is a group of metabolic disorders displaying unusually high blood glucose levels (Mealey *et al.*, 2007). The main forms are Type I, Type II and gestational diabetes. Type I diabetes or insulin-dependent diabetes is when the pancreas produces little or no insulin, whereas Type II diabetes is when there is resistance of the body to normal effects of insulin and the cells in the body loses capacity to produce enough insulin in the pancreas (i.e. insulin resistance). Women can develop hyperglycaemia during pregnancy and develop gestational diabetes (Casanova *et al.*, 2014).

Diabetes mellitus has been associated with varying degrees of systemic and oral complications. Over the years, it has been well-established that a two-way relationship exists between diabetes and periodontal disease. That is, poor glycaemic control is linked with an increased prevalence and severity of periodontal disease and severe periodontitis is correlated with compromised glycaemic control. Most of the research

conducted has been with Type II diabetes; however, Type I diabetes has similar effects (Lalla *et al.*, 2011, Casanova *et al.*, 2014; Sanz *et al.*, 2019).

The cross-sectional study by Gross *et al.*, (1994) utilizing 426 subjects showed that patients with diabetes were twice as likely to display AL in comparison to non-diabetic patients (Odds Ratio: 2:32). This study also found that regardless of age, diabetes was related to higher prevalence of periodontitis (Grossi *et al.*, 1994). Patients with long term diabetes are at greater risk of periodontitis; however metabolic levels also influence stability of periodontal disease. Oliver and Tervonen in their study in 1994 highlighted that people with diabetes with relatively good metabolic control had more periodontal pockets but not more tooth loss nor periodontal AL as compared to non-diabetics. People with diabetes who were poorly controlled with poor oral hygiene and extensive calculus had more periodontal disease and tooth loss than well-controlled and non-diabetics. Twenty seven percent of the poorly controlled people with diabetes in this study exhibited AL greater than 5 mm compared to only 10% in well-controlled subjects (Oliver *et al.*, 1994).

A definitive relationship was observed between metabolic control and complications associated with diabetes in the study by Tervonen and Karjalainen (1997). Their study assessed the healing response after initial phase therapy on 36 individuals with Type I diabetes. Patients with poor metabolic control and higher diabetic complications showed a greater extent of AL of more than 2 mm and were more likely to have the recurrence of pocket depths greater than 4 mm during the study. Whereas patients with good metabolic control and no diabetic complications demonstrated more favourable results (Tervonen *et al.*, 1997). Research by Chen *et al.*, (2010) found that subjects with Type II diabetes with increased mean PPDs displayed higher levels of glycated haemoglobin (HbA1c) and C-reactive protein (Chen *et al.*, 2010). In 2012, the same authors conducted a randomised control trial to test the effects of non-surgical periodontal therapy on clinical parameters, serum inflammatory parameters, and metabolic control in patients with Type II diabetes. This study suggested that periodontal therapy was associated with reduction of C-reactive protein levels in Type II patients (Chen *et al.*, 2012). Similarly Quintero *et al.*, reported that periodontal therapy can result in reduction of HbA1c levels in subjects with diabetes mellitus (Quintero *et al.*, 2018)

1.3.3 Other Risk Factors

There are several other risk factors that have been associated with periodontal disease. Non-modifiable factors such as age, gender, race and gene polymorphisms have been associated with this disease.

Aging is commonly related to periodontal disease with many epidemiological studies showing that more AL and bone loss is observed in older age groups in comparison to younger age groups (Grossi *et al.*, 1995; Grossi *et al.*, 1994; Streckfus *et al.*, 1999). The grading in the 2017 classification includes the assessment of bone loss relative to the patient's age as well as severity of clinical attachment loss (Tonetti *et al.*, 2018).

Inherited factors have been related to destructive periodontal disease; however more large scale, controlled studies are required (Genco *et al.*, 2013; Nunn, 2003). There are some genetic disorders that impact greatly on the periodontium. Patients with Down Syndrome have shown to have a higher prevalence and severity of periodontal disease with AL beginning in adolescents (Ram *et al.*, 2011). Pocket formation and severe inflammation is also found with patients with Papillon-Lefèvre syndrome. AL and alveolar bone loss occur at a rapid rate with loss of both primary and permanent dentition at a very young age (Khocht *et al.*, 2014; Vieira *et al.*, 2014).

Different ethnic groups may also display varying degrees of periodontal destruction. Ethnicity, however, is difficult to relate to periodontal disease, as other factors such as socio-economic status and genetic risk factors may also be contributory (Russell, 1962).

Young males and females and older males with low calcium intake have shown to have an increased risk of periodontal disease (Nishida, 2003). Occlusal discrepancies are also considered modifiable risk factors (Nunn *et al.*, 2001). The 2017 classification incorporates factors including primary occlusal trauma, secondary occlusal trauma and orthodontic forces (Fan *et al.*, 2018). Factors that influence plaque retention such as "prosthesis design", "fabrication", "materials", "tooth anatomic factors", "root abnormalities and fractures" and "tooth relationships" are also considered (Ercoli *et al.*, 2018). Tooth anatomy such as cervical enamel projections, developmental grooves

and tooth position are important factors to consider as they have been related to furcation involvement and increase in PPDs (Lim *et al.*, 2016; Matthews *et al.*, 2004).

Obesity, osteopenia/osteoporosis, Human Immunodeficiency Virus (HIV) and psychosocial factors, such as stress, have also been associated with periodontal disease. The meta-analysis on the relationship between obesity and periodontitis reported that the probability of periodontitis in patients that are obese compared to those who are not is between 50-80% (Chaffee *et al.*, 2010; Martinez-Herrera *et al.*, 2017; Suvan *et al.*, 2011). However, more understanding of the biological mechanisms underpinning the relationship between obesity and periodontitis is required. Genco *et al.*, (1999) linked stress, coping behaviours and depression to severe periodontal disease in adults. Stress was linked with greater AL and bone loss in these individuals (Genco *et al.*, 1999). De Marco in 1976 found that young men in the Vietnam War presented with severe vertical bone loss in the posterior areas with no obvious local factors and no evidence of bone loss prior to the war, although no definitive cause and effect relationship could be established (De Marco, 1976). A systematic review by Peruzzo *et al.*, (2007) summarized the association between chronic periodontal disease and stress and found a positive correlation between stress/psychological factors and periodontal disease (Peruzzo *et al.*, 2007).

The role of specific microbiota as risk factors for periodontitis has been researched extensively in the recent years and will be discussed in more detail under “Microbiology of Periodontal Disease”.

1.4 Clinical Parameters Associated with Periodontal Disease

Periodontal disease is characterized by clinical, inflammatory and radiographic changes. It is important that periodontal examinations systematically describes these changes, however, the cyclical nature of periodontal disease progression can sometimes make the use of clinical parameters complex (Socransky *et al.*, 1984).

The clinical diagnosis of periodontal disease has remained the same for the past few decades. Alterations in gingival colour, texture, consistency, periodontal pocket depths, gingival recession, tooth mobility, furcation involvement and BOP are recorded during the comprehensive periodontal assessment (Lindhe *et al.*, 2008). It is important that clinical parameters are reliable, easy to use, inexpensive, objective and must be able to be used widely (Greenstein, 1984). It will be also highly beneficial for clinicians if there was more information about the key predictors of periodontal treatment outcomes.

Significant reduction of clinical signs of gingival inflammation, reduction in PPDs and stabilization or gain of clinical attachment and progress toward the reduction of clinically detectable plaque to level compatible with gingival health are the outcomes of periodontal therapy as described by the AAP (Baelum *et al.*, 2016).

Periodontal pocket measurements are made from the gingival margin to the base of the sulcus and are measured in millimetres using a periodontal probe. The assessment of the periodontal pocket in relation to the CEJ is important to evaluate the periodontal attachment level (Lindhe *et al.*, 2008). In healthy sites, periodontal pocket depths range from 1-3 mm. Pocket depths greater than 4 mm were considered diseased, however, gingival swelling and pseudo-pockets need to be taken into consideration. Increase in probing pockets depths caused by destruction of the supporting tissue and bone, resulting in apical migration of the junctional epithelium below the CEJ is considered a true pocket. Rates of progression of disease as well as disease activity often associated with increase in pocket depth are also important factors to consider when diagnosing and treating periodontal disease (Armitage, 1999). Haffajee *et al.*, (1991) demonstrated that individuals with a mean baseline pocket depth or AL of >3 mm were

four times more likely to show AL compared to individuals with shallow pockets and minimal AL (Haffajee *et al.*, 1991a).

Errors can occur when recording probing depths. Factors such as different probing forces, variation in diameter of probe tips and gingival inflammation can result in differences in measurement (Grossi *et al.*, 1996; Lang *et al.*, 1996). However, Badersten *et al.*, (1984) demonstrated that by using a ± 1.0 mm standard deviation, approximately 90% of the probing measurements could be replicated. This can be done by using the standard deviations of the differences between duplicate measurements for pooled sites as a guideline. A difference between a pair of pre- and post-therapy recordings that exceeds 2 standard deviations would theoretically decrease the chances of making a false claim to below 5% (Badersten *et al.*, 1984d; Haffajee *et al.*, 1983).

BOP is one of the indicators used for diagnosis of inflamed gingival tissues and is associated with inflammatory cell infiltrate. BOP is normally assessed by a gingival bleeding index. A probe is gently inserted to the base of the pocket. If bleeding is noted after removal of probe, the site is considered positive for BOP (Ainamo *et al.*, 1975; Lindhe *et al.*, 2008). Bleeding may reflect histological, bacterial and clinical changes associated with periodontal disease; however, it is difficult to use bleeding as an indicator between stable and progressive periodontal lesions. Lang *et al.*, (1986) in a retrospective study found that BOP can be a useful prognostic indicator in clinical diagnosis for patients in the maintenance phase; however, it has limited use and needs careful interpretation (Lang *et al.*, 1986). Consequently, absence of bleeding is a more reliable marker of health of gingival tissues (Lang *et al.*, 1990). Variables such as the width of the probe, the angle in which the probe is inserted and even the pressure applied when probing must be taken into consideration when assessing BOP (Greenstein, 1984).

The presence of suppuration on probing is recorded during a comprehensive periodontal examination. The diagnostic predictability of suppuration on probing was found to be limited (20%) in the Badersten *et al.*, (1990) study as the presence of suppuration was infrequent during the study period (Badersten *et al.*, 1990). Similarly

Kaldahl *et al.*, (1990) also found that suppuration was a weak prognostic indicator of AL during a two-year maintenance period (Kaldahl *et al.*, 1990).

Haffajee *et al.*, (1991) showed that the greater the percentage of sites with visible plaque and bleeding upon probing, the greater the likelihood of subsequent AL (Haffajee *et al.*, 1991a). A retrospective study including 445 patients, found that in patients on supportive periodontal maintenance for at least 5 years, increased mean BOP was related to disease severity and instability of the periodontium and was not related to their smoking status (Ramseier *et al.*, 2015).

Furcation assessment from all entrances in multi-rooted teeth as well as the assessment of tooth mobility are important clinical diagnostic information that is required for diagnosis and treatment of periodontal disease. However, it is important to note that tooth mobility may not always be associated with plaque-associated periodontal disease. Other factors such as occlusal trauma, hypermobility, periapical lesions may also be linked (Lindhe *et al.*, 2008).

1.5 Radiographic Assessment

Dental radiographs are important diagnostic tools used as an adjunct in periodontal assessment and diagnosis and assist the clinician in identifying the different types of bone loss. Commonly used dental radiographs are panoramic radiographs and intraoral radiographs. These radiographs can be used to assess the level and pattern of alveolar bone destruction but also provide information on the lamina dura, periodontal ligament space, un-erupted or impacted teeth, furcation involvement, presence of calculus, restorations, shape of roots and any periapical issues present. Panoramic radiographs usually provide an overall view of the dentition, however more detailed information of specific areas can be obtained using intra-oral radiographs. Cone beam computerized tomography (CBCT) can also be utilized as a tool for diagnosis and treatment planning for patients with periodontal disease. It provides a three-dimensional view (3-D) and enables the clinician to view anatomy including furcation areas and osseous defects (du Bois *et al.*, 2012).

Nonsurgical periodontal therapy can result in osseous changes in the periodontium and valuable information can be obtained if these osseous changes can be tracked. High quality radiographs with minimal distortion are essential for accurate diagnosis, however, one of the limitations of conventional radiography is that it provides two-dimensional information on three-dimensional structures and there are issues with validity, precision and accuracy. In addition, there is superimposition of structures or bone defects which makes diagnosis difficult. However, many methods have been used to reduce the errors associated with conventional radiography techniques.

The use of paralleling film holding devices, and standardizing parameters such as film position, x-ray beam angulation and focal-film distance are some of the methods used to reproduce radiographic images. Hamanaka *et al.*, (2013) found that the use of occlusal splints and film holders provided a consistent reproduction of radiographic images (Hamanaka *et al.*, 2013). Customised bite blocks using polyether impression material were constructed and film holders were used to standardize radiographs taken in the Schmidt *et al.*, (1988) study. However, reproducibility of the radiographic images was not possible after two years due to deterioration of the impression material and tooth movement (Schmidt *et al.*, 1988).

CBCT radiography was found to be superior to conventional radiography and was able to detect all intraosseous defects, compared to only 67% when using conventional radiography (Vandenberghe *et al.*, 2007; Vandenberghe *et al.*, 2008). However, this cannot be used routinely as radiation dose to patients needs to be taken into consideration (du Bois *et al.*, 2012).

Digital radiography, which is more commonly used now, is a more efficient way of taking radiographs. It is less time consuming and there is lower radiation exposure to the patient. It also comes with software packages that include measurement tools and tools that can adjust the radiographic image (du Bois *et al.*, 2012; Mol, 2004).

Different methods have been used to track alveolar bone changes following periodontal therapy. Linear measurements from the CEJ to the alveolar crest have been utilized in studies (Nibali *et al.*, 2015b; Nibali *et al.*, 2011). However, the limitation

of using linear measurements is that underestimation of the changes in alveolar bone can occur (Toback *et al.*, 1999; Tonetti *et al.*, 1993).

Computer-assisted densitometric image analysis (CADIA) which includes use of digital subtraction radiography was used in the study by Hwang *et al.*, (2008). Changes in radiographic density of the coronal, middle and apical portions of defects were compared between radiographs taken at baseline and follow up (f/up) appointments. This study showed that 83% of the test regions recorded an increase in radiographic bone density following SRD (Hwang *et al.*, 2008). Studies have also used digital radiography to measure the vertical defect angle in infrabony defects following periodontal therapy (Nibali *et al.*, 2015b; Nibali *et al.*, 2011). Radiographic data following periodontal therapy are usually expressed as mean bone levels per subject or group of subjects or even as percentage of sites with radiographic bone loss or gain (Lindhe *et al.*, 2008).

1.6 Microbiology of Periodontal Disease

1.6.1 Non-Specific and Specific Plaque Hypothesis

Studies in the 1960s suggested that dental plaque was associated with periodontal disease and accumulation of dental plaque accompanied by reduced host resistance triggered periodontal disease destruction. According to the non-specific plaque hypothesis, bacteria colonizes the gingival crevice and forms plaque in the absence of oral hygiene. It was believed that plaque bacteria had various virulent properties to initiate gingival inflammation and periodontal destruction. The specific species of bacteria were not considered significant; with severity of inflammation related to the quantity of plaque (Loe *et al.*, 1965; Theilade, 1986).

However, periodontal destruction was not found to be related to the amount of plaque, it was often localised and in some cases patients with minimal destruction had rapid periodontal breakdown (Loë *et al.*, 1978). Improvements in anaerobic culture techniques provided valuable information on additional bacteria found in mature plaque. Previous research has established that differences existed between the

composition of biofilms between periodontally healthy or diseased sites or subjects (Teles *et al.*, 2008). This was seen particularly in studies related to localised aggressive periodontitis where there was minimal plaque accumulation and inflammation; however, severe connective tissue loss and bone loss was seen.

A. actinomycetemcomitans was found to be the potential etiological factor for localised juvenile periodontitis (Slots, 1976; Tanner *et al.*, 1979). The black-pigmented and fusiform *Bacteriodes* were implicated in cases of chronic periodontitis (Slots, 1977b; Tanner *et al.*, 1979). These findings led to the specific plaque hypothesis which supported the idea that specific bacteria caused disease (Loesche, 1976) and led to the further investigations into periodontal pathogens responsible for different periodontal conditions (Dzink *et al.*, 1988; Dzink *et al.*, 1985; Haffajee *et al.*, 1988b; Mombelli, 1996; Moore *et al.*, 1994; Slots *et al.*, 1985; Socransky *et al.*, 1988; Socransky *et al.*, 1987). Specificity of bacterial species and their role in periodontal disease and health became the focus of research. Several putative periodontal pathogens were identified and in 1996 at the World Workshop in Periodontics. *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia* were regarded as periodontal pathogens (Anonymous, 1996).

However, there were many difficulties faced in trying to determine the role of specific bacteria in relation to periodontal disease and progression. These included the diversity of the subgingival microbiota, some of which are still unculturable; the problems associated in obtaining a sample that is representative of the subgingival microflora; identifying sites that have active disease and the fact periodontal disease is a mixed infection and a combination of bacteria may be responsible (Teles *et al.*, 2013). A set of criteria to help define subgingival periodontal pathogens, developed by Socransky and Haffajee in 1992 provided a clearer understanding of periodontal microflora and their association with periodontal disease and progression (Socransky *et al.*, 1992). Even though there have been variations in these theories, periodontal disease can be described by the combination of these theories and the role of bacteria in the initiation and progression of periodontal disease is certain.

The new model of pathogenesis moves away from the concept that single or complexes of bacteria such as the “red complex” are responsible for periodontal disease but is initiated by a broadly-based dysbiotic, synergistic microbiota. This

concept underpins the knowledge that diverse microbiota and compatible microbiota are present in the gingival crevice in equilibrium with the host. Pathogenicity and overgrowth are controlled by the host. Microbial communities between individuals and site to site can vary over time. Pathogens such as *P. gingivalis* can elevate the virulence of the microbial community and can in turn cause impairment of the host response resulting in increases in the dysbiotic community and eventually resulting to disruption of tissue homeostasis which leads to destruction of the periodontal tissues (Hajishengallis *et al.*, 2012a; Hajishengallis *et al.*, 2012b).

1.6.2 Maturation of Plaque

Plaque collected after 24 hours consists of mainly *Streptococci sangius* and some *Actinomyces species*. However, as the plaque matures, Gram positive rods which are initially found in low numbers are outnumbered and replaced by Gram positive filaments namely *Actinomyces species*. Gram positive facultative rods and filaments co-aggregate, creating anaerobic conditions and resulting in the adherence of the Gram-negative species such *Veillonella*, *Fusobacterium* and other Gram-negative species. This increase in colonization of the Gram negative species increases the pathogenicity of the biofilm (Listgarten *et al.*, 1975).

Dental calculus can be described as mineralised dental plaque. Supragingival calculus formation is not only dependent on bacterial plaque but also on salivary gland secretion, hence it can be found adjacent to excretion ducts of salivary glands. Subgingival calculus is found apical to the gingival margin and can be detected using tactile exploration. Both supragingival and supragingival calculus harbour bacterial plaque. Subgingival calculus in addition to bacterial plaque is mixed with gingival crevicular fluid and blood. Subgingival calculus is found in periodontal pockets usually extending to the CEJ and close to the base of the pocket; however, a band approximately 0.5mm usually coronal to the apical portion of the periodontal pocket is usually free of mineralised deposits (Lindhe *et al.*, 2008).

Dental calculus can be tenacious and adhere to the tooth and root surface, making debridement difficult. However, it has been demonstrated that surface roughness does not only contribute to gingivitis (Waerhaug, 1956). Dental calculus is usually covered

with viable bacterial biofilm making it difficult to establish whether the periodontal tissues are affected by the presence of calculus or biofilm. However, the presence of calculus deposits can amplify bacterial plaque deposits keeping them close to the tissues thereby contributing to microbial ecology and tissue response (Friskopp *et al.*, 1980). Past studies have supported that dental calculus is not a primary cause of periodontal disease but rather a secondary etiologic factor for periodontitis (Allen *et al.*, 1965; Listgarten *et al.*, 1973).

1.6.3 Change in Microflora from Health to Disease

During the shift from periodontal health to disease, there is a change from mostly Gram positive species to mostly Gram negative species (Marsh, 1994). Gram positive cocci bacteria prominent in healthy sites included *Streptococcus mitis*, *Streptococcus sanguis*, *Staphylococcus epidermidis*, *Rothia dentocariosa*, *Actinomyces viscosus*, *Actinomyces naeslundii*, and occasionally species of *Neisseria* and *Veillonella* (Socransky, 1977; Ximenez-Fyvie *et al.*, 2000a).

The *Actinomyces* species are found in both health and disease with the *Actinomyces* species seen in experimental gingivitis (Socransky, 1977). Past studies indicated that proportions of certain bacteria increase with gingivitis (Loe *et al.*, 1965; Theilade *et al.*, 1966). Long standing gingivitis was associated with 25% of Gram-negative bacteria and included species of *Veillonella*, *Campylobacter* and *Fusobacterium* as well as Gram negative fusiform rods namely *Bacteriodes ochraceus* (Socransky, 1977). Tanner *et al.*, (1998) in their 12-month longitudinal study found that *A. naeslundii*, *Campylobacter gracilis* (*C.gracilis*) and *T. forsythia* were the predominant species in gingivitis (Tanner *et al.*, 1998a).

The ecological progression of bacterial species in subgingival and supragingival plaque after 7 days of no oral hygiene in healthy and periodontitis subjects was analysed in the study by Teles *et al.*, in 2012. Subjects in periodontitis groups underwent SRP and subjects in the periodontally healthy group had prophylaxis with a rubber cup, paste and flossing. Supragingival plaque development was found to be similar in both groups. At day 1 and 2, supragingival plaque samples of both groups showed an increase in mean proportions of *Streptococcus mitis*, *Streptococcus oralis*,

E. corrodens and *Neisseria mucosa*. Elevated proportions of *S. mitis* and *N. mucosa* is in line with their role as early colonizers. At day 2, there was a significant increase in *Veillonella parvula* in periodontitis subjects but later at day 4 in healthy subjects. A more complex bacterial community was also observed following increase in proportion of *V. parvula*. There were significant increases in mean proportions of *C. gingivalis* in both groups at day 2 and 4. *C. rectus*, *Campylobacter showae*, *C. ochracea* and *Prevotella nigrescens* increased at days 4-7. Significant increases in *P. intermedia* and *C. gracilis* were observed in the periodontitis subjects but not in the healthy subjects, whereas the mean proportion of *C. sputigena* increased only in healthy subjects.

The development of subgingival plaque was found to be different in the two groups and much slower as compared to supragingival plaque development. *S. mitis* was first observed in increased proportions, followed by *V. parvula* and *C. gingivalis*. At day 7, *C. sputigena* and *P. nigrescens* were seen. Interestingly, changes began much later in the subgingival biofilm development in periodontitis patients as compared to periodontally healthy patients. However, by day 7, significant increases were seen in the periodontitis group. The orange complex bacteria namely *C. gracilis*, *P. intermedia* and *P. nigrescens* were higher in the periodontitis subjects suggesting a possible change towards more pathogenic bacteria. However, the proportions of the classic periodontal pathogens showed no significant increase in both groups and there was a decrease in proportions of *E. nodatum*, *P. gingivalis*, *T. forsythia* and *T. denticola* in the periodontitis group suggesting that these bacteria may take even longer to return to pre-treatment levels (Teles *et al.*, 2012).

1.6.4 Criteria for Defining Periodontal Pathogenic Bacteria

Over seven hundred species of bacteria have been identified in the oral cavity (Moore *et al.*, 1994; Paster *et al.*, 2001). More than four hundred of these species have been identified in the periodontal pocket and the remaining 300 found in other areas of the oral cavity such as the tongue, oral mucous membrane, carious and endodontic infections (Paster *et al.*, 2006). There is much diversity in bacterial colonization in different people and individuals are thought to have about 100-200 of these 700 bacterial species. There are a few species that are seen in most individuals and

colonize many areas such as the *Streptococcus species* and *Veillonella species* (Paster *et al.*, 2006). The development of more advanced technologies such as the DNA-DNA hybridization techniques and real-time Polymerase Chain Reaction (PCR) have made it possible to examine a wider variety of bacterial species and allowing us to isolate, characterize and name over 250 of these species (Ashimoto *et al.*, 1996; Paster *et al.*, 2006; Socransky *et al.*, 1994a).

Periodontal pathogens are crucial in inflammatory periodontal disease; however although pathogens are necessary, they may not be sufficient for disease activity to occur (Haffajee *et al.*, 2006a; Kinane, 2001; Socransky *et al.*, 1992). There are more than 700 bacterial taxa colonizing the oral cavity, however, only 10-20 species have been linked to the pathogenesis of periodontal disease (Holt *et al.*, 2005; Socransky *et al.*, 1994a).

In 1992, Socransky and Haffajee, hypothesised that specific bacteria are linked to periodontal disease progression; however, disease initiation and progression involves an interplay of number of factors based on Koch's postulates:

1. The bacteria must be virulent clonal type. Virulence is defined as set of unique properties which allows bacteria to colonize a target organ; defend itself from the host and cause tissue destruction or damage (Holt *et al.*, 2005; Socransky *et al.*, 1991a).
 2. The bacteria must have chromosomal and extra-chromosomal genetic factors to initiate disease.
 3. The host must be susceptible to the pathogen.
 4. The bacteria must be located in the right place and the numbers sufficient to exceed the host's threshold.
 5. Other bacterial species must foster, or not inhibit the disease process.
 6. The local environment must be conducive.
- (Socransky *et al.*, 1992).

Koch's postulates have been extended to include association, elimination, animal pathogenicity, host responses, and formation of virulence factors (Socransky *et al.*, 1992).

1. Association: the suspected species should be found in higher numbers in active and diseased sites and low numbers in healthy sites or in sites showing progression.
2. Elimination: treatment should decrease the levels of the pathogens and stop disease progression. Sites that still harbour bacteria will lead to progression in that site or subject.
3. Elevated immunological response should be found.
4. The ability of the species to produce virulence factors.
5. The bacteria should have produced biochemical properties which is an indicator of the species to contribute to disease progression.

1.6.5 Development of Periodontal Microbial Complexes

The microbiology of periodontal disease has been the focus of research for several decades. In the earlier years, limitations in isolation and culture techniques did not allow the identification of the more fastidious bacteria involved in periodontal disease. However, in the recent years, with advancements in molecular techniques, the knowledge of the composition of the subgingival microbiota has improved significantly allowing the examination of larger samples and providing valuable information on the unculturable segment of the microbiota as well as the role of viruses in the etiology of periodontal disease (Teles *et al.*, 2013).

However, culture techniques used in the past, have provided important information and contributed significantly to the development of the periodontal microbial complexes. Initial studies examined bacterial species associated with gingivitis, localised juvenile periodontitis and chronic periodontitis. Pathogens such as *P. gingivalis*, *T. forsythia* and *A. actinomycetemcomitans* were identified (Moore *et al.*, 1994; Slots, 1979; Socransky *et al.*, 1994a).

Tanner *et al.*, in 1979, found that bacteria in the apical area of subjects with advancing periodontitis lesions, were mainly Gram-negative cocci and all were members of the *Veillonella* group. Gram positive rods were found in large numbers in minimal disease sites whereas Gram negative rods were found in deep destructive sites (Tanner *et al.*, 1979). In rapidly progressive periodontitis Gram negative fusiform rods specifically,

Bacteroides melaninogenicus ss. *asaccharolyticus*, and spirochetes were identified. However, other bacteria were also found and included monotrichously flagellated organisms and "corroding" bacteria, as well as an undescribed genus of anaerobic vibrios, "corroding" *Bacteroides*, *E. corrodens*, *F. nucleatum*, and a newly described group of fusiform shaped "gelatin loving" *Bacteroides* (Socransky, 1977).

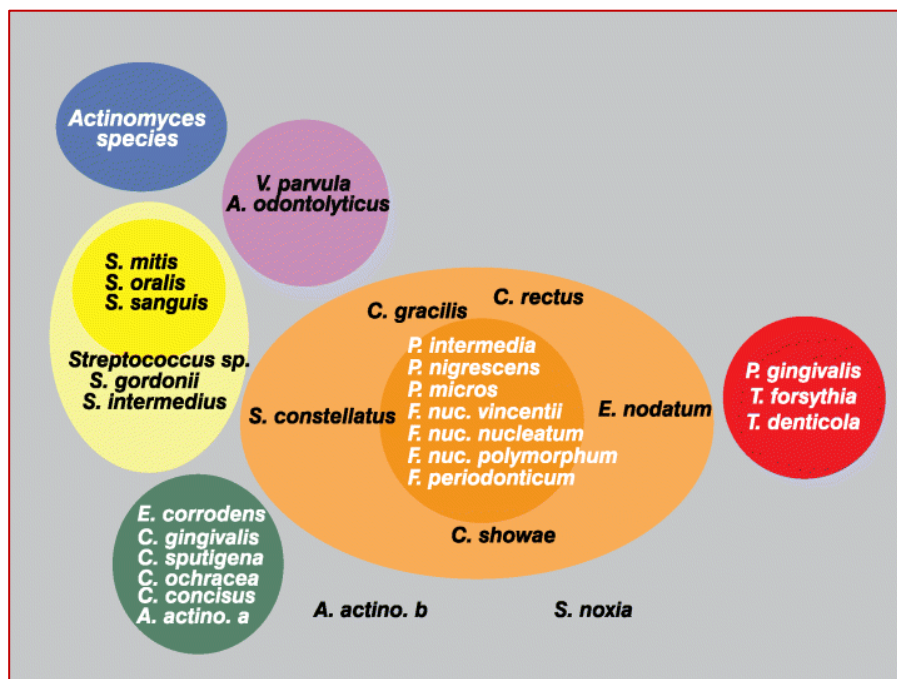
Socransky *et al.*, in 1988 found that a combination of bacteria namely *F. nucleatum*, *B. forsythus* and *Wolinella recta* or a combination of *Bacteriodes gingivalis*, *Bacteriodes intermedius*, *Streptococcus intermedius* were found in highest proportions in sites with greater than 3 mm of AL (Socransky *et al.*, 1988). The longitudinal prospective study by Haffajee *et al.*, (1991) did not identify clusters of bacteria, however, supported the concept that pathogens do not live in isolation. Levels of *Bacteriodes gingivalis* and *Wolinella recta* were found elevated in moderate and deep pockets and *P. micros* were identified in deep progressing pockets. *B. forsythus* was found at higher levels in shallow and moderate progressing pockets (Haffajee *et al.*, 1991b).

There were suggestions that the bacteria that inhabit the periodontal pockets may be found in groups or as complexes of bacteria. Socransky *et al.*, (1998) highlighted that different complexes of bacteria may inhabit different periodontal pockets and that a better understanding of oral diseases will be obtained by focussing on the consortia of bacteria rather than the individual species. However, there was still some uncertainty on how these complexes develop (Socransky *et al.*, 1998). Socransky and Haffajee in 1998 provided a structure to improve the understanding of the intricate ecology of dental plaque based on the analysis of the microbial profiles of over 13,000 plaque samples from 185 subjects. Cluster analysis and community ordination techniques were utilized to examine the relationship among 32 subgingival bacterial species and related these complexes to clinical parameters in periodontal disease. This framework has provided some guidance over the years in diagnosis and treatment of periodontal disease (Socransky *et al.*, 1998).

The microbes were categorized into six main groups as illustrated in the Figure 1.4 (Socransky *et al.*, 2005). The data were adapted and reorganized from the original representation of the complexes showing it as a pyramid (Socransky *et al.*, 2002a).

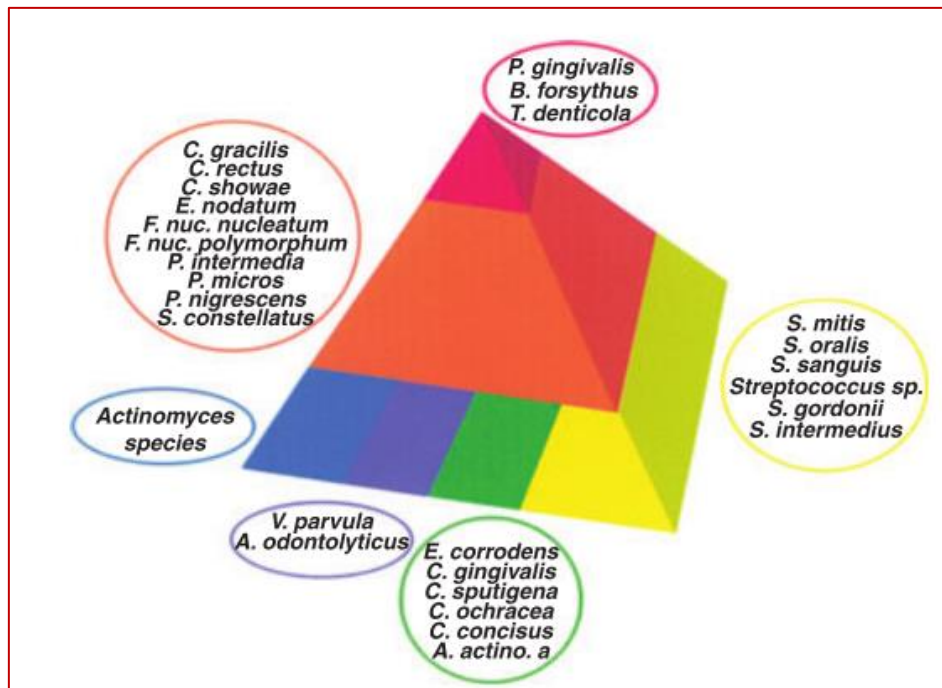
Each group builds the base for colonization by the next level as demonstrated in Figure 1.5 (Socransky *et al.*, 2002a). Based on the 16SrDNA sequence analysis, *B. forsythus* was reclassified as the first species of a new genus *Tannerella* as *Tannerella forsythensis* *corrigen.,gen.nov.,comb.nov* (Sakamoto *et al.*, 2002). Its current proposed name of *Tannerella forsythia* will be used (Maiden *et al.*, 2003).

Figure 1. 4 Diagrammatic representation of the relationships of species within and between the microbial complexes. Updated from (156) with *B. forsythus* changed to *T. forsythia*.



Reproduced from (Socransky *et al.*, 1998)

Figure 1. 5 A pyramid representation of bacterial groups



Reproduced from(Socransky *et al.*, 2002a)

Specific species of *Actinomyces*, the yellow complex, green complex and purple complex were thought to be early colonizers of the tooth surface whose growth precedes the growth of the red and orange complexes. The red and orange complexes consist of pathogens that are thought to be major causative agents of periodontal disease (Lindhe *et al.*, 2008; Socransky *et al.*, 1998). Higher proportions of red and orange complex bacteria were found with subgingival proportions of the red complex twice of that observed supragingivally (Ximenez-Fyvie *et al.*, 2000b). Studies have found that the red complex was rarely found in the absence of the orange complex suggesting that the orange complex may precede the colonization of the red complex (Socransky *et al.*, 1998).

In addition, a strong relationship was observed between the pocket depths and the red complex. In subgingival plaque samples, increasing numbers of red complex bacteria are found in diseased sites; however these bacteria are also found in supragingival plaque suggesting that supragingival plaque can harbour bacteria for recolonization

(Ximenez-Fyvie *et al.*, 2000b). The orange complex also showed a significant association with increasing pocket depth and BOP (Socransky *et al.*, 1998).

The prevalence of *B. forsythus*, *P. gingivalis* and *A. actinomycetemcomitans* was investigated among 21 adult periodontitis patients, 8 patients with rapidly progressive periodontitis and 15 healthy persons. The prevalence and proportion of *T. forsythia* and *P. gingivalis* were significantly associated with clinical parameters suggesting that these two bacteria are closely associated with periodontal breakdown. *T. forsythia* was found to be tenfold higher during the active phase than static phase of periodontitis, whereas *P. gingivalis* doubled during the active phase. This may suggest that *T. forsythia* may be involved in the process of tissue destruction such as deepening of the pocket and AL (Yano-Higuchi *et al.*, 2000).

For the current study, eleven periodontal pathogens were examined including: *A. actinomycetemcomitans*; *P. gingivalis*; *T. forsythia*; *T. denticola*; *P. intermedia*; *P. micros*; *F. nucleatum*; *C. rectus*; *E. nodatum*; *E. corrodens* and *Capnocytophaga sp.* (*sputigena*; *gingivalis*; *ochracea*). The bacteria were also examined in their respective complexes, namely red, orange, orange-associated and green complex.

1.6.5.1 *A. actinomycetemcomitans*

A. actinomycetemcomitans is a non-motile, facultative anaerobic co-bacillus bacterium (Henderson *et al.*, 2002; Zambon *et al.*, 1983). *A. actinomycetemcomitans* has been regarded as one of the main etiological agents in aggressive periodontitis (Faveri *et al.*, 2009; Fine *et al.*, 2007; Henderson *et al.*, 2002; Zambon *et al.*, 1983). However, it has also been found in adult periodontitis (Brigido *et al.*, 2014; Christersson *et al.*, 1992; Grossi *et al.*, 1994; Haffajee *et al.*, 1988b; Holt *et al.*, 2005; Kornman *et al.*, 1991; Mombelli *et al.*, 1994a; Riggio *et al.*, 1996; Rodenburg *et al.*, 1990; Savitt *et al.*, 1984). This pathogen has also been detected in periodontally healthy patients but also found to increase in disease (Fives-Taylor *et al.*, 1999; Slots *et al.*, 1999; Tanner *et al.*, 1998a).

A. actinomycetemcomitans has also been detected in deep sites (Ebersole *et al.*, 1994; Mombelli *et al.*, 1994a; Slots *et al.*, 1980; Socransky *et al.*, 1991a; Tanner *et al.*, 1979) with many studies reporting the difficulty to eradicate this pathogen from deep periodontal pockets (Bragd *et al.*, 1987; Renvert *et al.*, 1990). This pathogen has also been found in increasing levels in progressing or active sites (Dzink *et al.*, 1983; Dzink *et al.*, 1988; Dzink *et al.*, 1985; Slots, 1986; Slots *et al.*, 1986; Tanner *et al.*, 1987b).

Reduction of *A. actinomycetemcomitans* following SRD has been reported in many studies (Darby *et al.*, 2005; Flemmig *et al.*, 1998a; Listgarten *et al.*, 1991; Maiden *et al.*, 1991; Mombelli *et al.*, 1994a, 1994b; Nieminen *et al.*, 1996; Preber *et al.*, 1995; Renvert *et al.*, 1997; Renvert *et al.*, 1990; Rosenberg *et al.*, 1993; Shiloah *et al.*, 1994; Sigurdsson *et al.*, 1994; Wikström *et al.*, 1993). Studies have shown that subjects with localised juvenile periodontitis have an increased serum antibody response to *A. actinomycetemcomitans* (Altman *et al.*, 1982; Ebersole *et al.*, 1980; Ebersole *et al.*, 1983; Ebersole *et al.*, 1982; Tsai *et al.*, 1981) and with successful treatment of these subjects, the species reduced in numbers or were eliminated. However, if treatment was not successful, then numbers of these species remained high in treated sites (Christersson *et al.*, 1985; Haffajee *et al.*, 1984; Kornman *et al.*, 1985; Mandell *et al.*, 1986; Slots *et al.*, 1983).

A. actinomycetemcomitans can be grouped into six serotypes (a-f) based on the polysaccharide antigen on the cell surface. A systematic review by Brigido *et al.*, (2014) found that the dominant serotypes are a, b and c, with d and e being rarer. More research needs to be conducted on the new serotype f (Brigido *et al.*, 2014). Studies conducted on US and Finnish subjects found that serotype b were often associated with localized juvenile periodontitis (Mombelli *et al.*, 2002). Haffajee *et al.*, (1991), found that the mean percentage of serotype b of *A. actinomycetemcomitans* was higher in patients with active disease (Haffajee *et al.*, 1991b). Results from different parts of the world varied suggesting that *A. actinomycetemcomitans* differs in terms of distribution patterns among subjects of different ethnicity and geographic locations. In addition, knowledge between the different serotypes and various periodontal conditions is still very ambiguous (Brigido *et al.*, 2014).

This bacteria is an opportunistic pathogen that produces a variety of virulence factors such as lipopolysaccharide, leukotoxin, collagenase, protease, cytothelial distending toxins and fibroblast inhibition factor (Curtis *et al.*, 2005; Kachlany, 2010; Popova *et al.*, 2013). The highly leukotoxic strains produce more toxin and interferes with the host defence mechanisms (Brogan *et al.*, 1994). *A. actinomycetemcomitans* destroys both hard and soft tissues and also disrupts host tissue repair after infection. In animal studies, *A. actinomycetemcomitans* was found to induce disease in gnotobiotic rats and cause subcutaneous abscesses in mice (Chen *et al.*, 1991; Irving *et al.*, 1975; Wray *et al.*, 1992).

1.6.5.2 The Red Complex

The red complex bacteria colonise later in biofilm development and is considered the most significant complex. Increase in prevalence and numbers have been related to increase in clinical parameters associated with periodontal disease. The specific species considered periodontal pathogens found together in plaque samples include *P. gingivalis*, *T. denticola*, and *T. forsythia* (Holt *et al.*, 2005). *E. nodatum* was added to the red complex bacteria as it was shown to be closely associated with the periodontitis (Haffajee *et al.*, 2008b; Haffajee *et al.*, 2006a). However, for this study *E. nodatum* was analysed as part of the orange-associated complex.

Mineoka *et al.*, (2008) assessed the quantitative relationship between *P. gingivalis*, *T. forsythia* and *T. denticola* in the subgingival biofilm. In healthy sites, the numbers and frequency of all three bacteria occurring together were lower compared to sites with pocket depths greater than 4 mm and BOP. The presence of all three bacteria were also found to be linked to specific sites that were deteriorating suggesting that the development of periodontal disease may be influenced by the quantity and prevalence of *P. gingivalis*, *T. forsythia* and *T. denticola* in the subgingival biofilm (Mineoka *et al.*, 2008).

1.6.5.2.1 *P. gingivalis*

P. gingivalis is a Gram negative, anaerobic, asaccharolytic, non-motile rod species. It is a member of the black-pigmented *Bacteriodes* group and was previously known as *B. melaninogenicus*, *B. asaccharolyticus*, *B. gingivalis* and *Haemophilus melaninogenicus* (Haffajee *et al.*, 1994; Lindhe *et al.*, 2008).

Data from several studies have found *P. gingivalis* elevated in lesions of periodontitis (Albandar *et al.*, 1990; Choi *et al.*, 1990; Christersson *et al.*, 1992; Dahlen *et al.*, 1992b; Dzink *et al.*, 1988; Gmür *et al.*, 1989; Haffajee *et al.*, 1988b; Loesche *et al.*, 1985; Moore *et al.*, 1985; Moore *et al.*, 1983; Moore *et al.*, 1991; Papapanou *et al.*, 1993; Slots, 1977b, 1979, 1986; Slots *et al.*, 1986; Spiegel *et al.*, 1979; Tanner *et al.*, 1979; Tanner *et al.*, 1984; Zambon *et al.*, 1981). It has been detected in low numbers in health or gingivitis (Choi *et al.*, 1990; Moore *et al.*, 1985; Moore *et al.*, 1991; Savitt *et al.*, 1984; Slots, 1977a, 1977b; Slots *et al.*, 1978; Spiegel *et al.*, 1979; Tanner *et al.*, 1998a; White *et al.*, 1981; Zambon *et al.*, 1981). Studies have also found *P. gingivalis* to be more frequently detected in the destructive form of disease (Haffajee *et al.*, 1994; O'Brien-Simpson *et al.*, 2000; Takeuchi *et al.*, 2001; van Winkelhoff *et al.*, 2002). This pathogen has also been detected in higher numbers and frequency in active periodontal sites (Dzink *et al.*, 1988; Dzink *et al.*, 1985; Haffajee *et al.*, 1991c; Moore *et al.*, 1991; Slots *et al.*, 1986) and in subjects displaying periodontal disease progression (Albandar *et al.*, 1997). Griffen *et al.*, (1998) studied 130 patients with periodontitis and 181 healthy patients. *P. gingivalis* was detected in 79% of the periodontitis subjects and 25% of the healthy subjects. The odds ratio of being infected with *P. gingivalis* was 11.2 times (95%CI: 6.5-19.2) greater in the periodontitis group as compared to the healthy group (Griffen *et al.*, 1998). van Winkelhoff *et al.*, (2002) reported that *P. gingivalis* and *T. forsythia* were most strongly associated with periodontal destruction with an odds ratio of 12.3 and 10.4 respectively. *P. gingivalis* was found in 60% of the periodontitis subjects and in 10.6% of the healthy group (van Winkelhoff *et al.*, 2002).

Many studies have demonstrated a decrease in *P. gingivalis* after SRD (Ali *et al.*, 1992; Haffajee *et al.*, 1997b; Haffajee *et al.*, 1995; Hellstrom *et al.*, 1996; Mombelli *et al.*, 1995; Preber *et al.*, 1995; Renvert *et al.*, 1997; Renvert *et al.*, 1990; Simonson *et al.*, 1992b; Slots *et al.*, 1985; Socransky *et al.*, 1993; Wikström *et al.*, 1993). However,

recurrent lesions still harboured this species (Bragd *et al.*, 1987; Choi *et al.*, 1990; Nakagawa *et al.*, 1990; van Winkelhoff *et al.*, 1988). *P. gingivalis* has a strong association with probing depths with sites harbouring *P. gingivalis* alone or in combination with the other red complex bacteria showing the deepest mean probing depths (Socransky *et al.*, 1998). Using DNA probes to detect and quantify *P. gingivalis* in 25 subjects with periodontal disease and 25 healthy subjects, O'Brien-Simpson *et al.*, (2000) found that almost 46% of the sites examined were positive for *P. gingivalis* in the periodontal disease group compared to 10% in the healthy group. This study also found that statistically significant positive associations between percentage of sites positive for *P. gingivalis* and mean pocket depths, mean AL and percentage of sites with BOP (O'Brien-Simpson *et al.*, 2000). Similarly, Hamlet *et al.*, in 2001 also reported a strong association with pocket depth and *P. gingivalis*. This study included 6,030 plaque samples taken from 504 subjects. *P. gingivalis* was 15.3 times more likely to be detected in pockets greater than 5 mm as compared to pocket depths less than 3 mm (Hamlet *et al.*, 2001).

This pathogen produced increased systemic and local immune responses in subjects with different types of periodontal disease (Altman *et al.*, 1982; Choi *et al.*, 1990; Curtis *et al.*, 1991; Ebersole *et al.*, 1986; Ebersole *et al.*, 1987; Ebersole *et al.*, 1982; Laosrisin *et al.*, 1990; Lopatin *et al.*, 1992; Mahanonda *et al.*, 1991; Mooney *et al.*, 1993; Vincent *et al.*, 1985; Whitney *et al.*, 1992; Wilton *et al.*, 1992).

Several virulence factors have been reported for *P. gingivalis* including gingipains, fimbriae, hemagglutinin, capsule and lipopolysaccharides. This species produces collagenase, proteases including those that destroy immunoglobulins, endotoxins, fatty acids, ammonia (NH₃), hydrogen sulphide (H₂S), fibroblast inhibitory factor, factors that negatively affect neutrophils, bone-resorption inducing factor and chemotactic activity (Haffajee *et al.*, 1994). The observations made in the Ogawa *et al.*, (1989) study was that in advanced periodontitis lesions about 5% of the plasma cells produce antibody to the fimbria of *P. gingivalis* (Ogawa *et al.*, 1989).

Majority of strains of *P. gingivalis* are encapsulated, which can reduce host's response, and result in the bacterium surviving for a prolonged period. In chronic inflammatory conditions, this long-term persistence of the pathogen may produce a low grade, inflammatory response (Holt *et al.*, 2005; O'Brien-Simpson *et al.*, 2004). The ability of

P. gingivalis to invade gingival epithelial tissues has been demonstrated in *in-vitro* studies (Duncan *et al.*, 1993; Lamont *et al.*, 1992; Sakanaka *et al.*, 2016; Sandros *et al.*, 1993; Sandros *et al.*, 1994).

In animal studies, *P. gingivalis* has been shown to induce disease in gnotobiotic rats (Evans *et al.*, 1992b; Irving *et al.*, 1975). In studies involving dogs, sheep and monkeys, *P. gingivalis* organisms were found to be strongly associated with destructive periodontal disease (Ebersole *et al.*, 1991; Holt *et al.*, 1988; Kornman *et al.*, 1981a; Kornman *et al.*, 1981b; McCourtie *et al.*, 1990). Some animal studies have reported decrease in disease severity following immunization with the whole organism or specific antigens (Evans *et al.*, 1992a; Evans *et al.*, 1992c).

1.6.5.2.2 *T. denticola*

T. denticola is a spirochete bacterium that colonizes the gingival crevice. It is a rapidly motile obligatory anaerobic bacterium that has been associated with the progression of periodontal disease. This bacteria is found more commonly in periodontally diseased sites than healthy sites and more frequently in subgingival than supragingival plaque (Riviere *et al.*, 1992; Simonson *et al.*, 1988). *T. denticola* can account for approximately 50% of the total bacterium in a periodontal lesion (Listgarten, 1976; Moore *et al.*, 1994). *T. denticola* is not an early colonizer and has been found to inhabit the deeper periodontal pockets (Kolenbrander *et al.*, 2002).

Previous research has demonstrated that SRD decreases the levels of spirochaetes including *T. denticola* (Cugini *et al.*, 2000; Haffajee *et al.*, 1997b; Listgarten *et al.*, 1981; Loesche *et al.*, 1992; Loesche *et al.*, 1985; Simonson *et al.*, 1992b). A number of studies have also reported that the reduction of *T. denticola* following treatment has been associated with clinical improvements (Cugini *et al.*, 2000; Simonson *et al.*, 1992b). In 1992, Simonsen *et al.*, found that reduction in probing depths following treatment was associated with a decrease in the *T. denticola* antigen. Though, in poorly responding sites, *T. denticola* antigen either stayed the same or increased as compared to pre-treatment levels, suggesting that this bacterium could be a valuable prognostic marker for periodontal disease recurrence (Simonson *et al.*, 1992b). This was confirmed in the Cugini *et al.*, (2000) study who showed a decrease in frequency

and levels of *T. denticola* after SRP correlated with decreasing pocket depths and BOP (Cugini *et al.*, 2000).

Spirochetes are found next to the epithelial lining of the periodontal pocket and may facilitate attachment of spirochetes to epithelial cells and invasion into adjacent tissues (Lindhe *et al.*, 2008). *T. denticola* possesses a number of virulence factors with its major virulence factors being its motility and chemotaxis which enables this bacteria to penetrate deep periodontal pockets and epithelial layers; rapidly colonize new sites; produce cytotoxic metabolites (Ishihara, 2010) and have synergistic relationships with other pathogens. Studies by Byrne *et al.*, (2009) and Mineoka *et al.*, (2008) found *P. gingivalis* and *T. denticola* together in subgingival plaque (Byrne *et al.*, 2009; Mineoka *et al.*, 2008). *P. gingivalis* and *T. denticola* are thought to have a synergistic relationship and are associated with the clinical signs of periodontal destruction and the developing biofilm (Holt *et al.*, 2005; Simonson *et al.*, 1992a).

T. denticola has the capability to form biofilms and cell-surface proteins to destroy the host defence mechanism and cause tissue destruction (Dashper *et al.*, 2011). The outer surface membrane is thought to allow *T. denticola* to adhere to the gingival crevice (Chan *et al.*, 2000). The major virulence factor for *T. denticola* is thought to be Dentilisin which allows for penetration of the epithelial cell layers by degradation of the intercellular adhesion proteins (Chi *et al.*, 2003). *T. denticola* has the ability to suppress the host responses to lipopolysaccharides which allows for the persistence of bacteria associated with periodontal disease (Dashper *et al.*, 2011). Animal studies have linked this species with development of periodontal disease in dogs (Nordhoff *et al.*, 2008).

1.6.5.2.3 *T. forsythia*

T. forsythia is the third member of the red complex as described by Socransky *et al.*, in 1998 (Socransky *et al.*, 1998). *T. forsythia* was first reported in the literature by Tanner *et al.*, (1979). It was originally identified as a member of the fusiform *Bacteriodes* species and was named *Bacteriodes forsythus* (Tanner *et al.*, 1979). It is an anaerobic, Gram negative, spindle shaped, pleomorphic rod and is commonly found in subgingival sites with *F. nucleatum* species (Lindhe *et al.*, 2008). Based on 16S rDNA analysis, *Bacteriodes forsythus* was reclassified by Sakamoto *et al.*, and

identified as a new genus, *Tannerella*, hence the name *Tannerella forsythia* (Sakamoto *et al.*, 2002).

T. forsythia has been found in higher numbers in sites of periodontal destruction than in gingivitis or healthy sites (Lai *et al.*, 1987). Studies have reported this bacteria in subgingival plaque of healthy children (Kimura *et al.*, 2002) and in periodontally healthy adults (Ximenez-Fyvie *et al.*, 2000a). Both cross sectional and longitudinal studies have identified *T. forsythia* in chronic and in aggressive periodontitis (Ali *et al.*, 1997; Choi *et al.*, 2000; Colombo *et al.*, 2002; Darby *et al.*, 2000; Darby *et al.*, 2001; Dzink *et al.*, 1985; Haffajee *et al.*, 1998; Haffajee *et al.*, 1994; Haffajee *et al.*, 1988c; Haffajee *et al.*, 1992; Kamma *et al.*, 1999; Kasuga *et al.*, 2000; Kumar *et al.*, 2003; Kumar *et al.*, 2005; Leys *et al.*, 2002; Moore, 1987; Moore *et al.*, 1994; Papapanou *et al.*, 1997a; Papapanou *et al.*, 2000; Tanner *et al.*, 1979; Tanner *et al.*, 1984; Trevilatto *et al.*, 2002; Umeda *et al.*, 1998; van Winkelhoff *et al.*, 2002; Ximenez-Fyvie *et al.*, 2000a; Yang *et al.*, 2004; Yano-Higuchi *et al.*, 2000). *T. forsythia* has also been associated with progressing lesions post-therapy (Edwardsson *et al.*, 1999; Haffajee *et al.*, 1988c; Heitz-Mayfield *et al.*, 2006; Huang *et al.*, 2003; Lai *et al.*, 1987; Shiloah *et al.*, 1998; Socransky *et al.*, 1993; Socransky *et al.*, 2002b; Winkel *et al.*, 1997). Many studies have also associated *T. forsythia* with early or initial periodontitis (Suda *et al.*, 2004; Tanner *et al.*, 1989; Tanner *et al.*, 1996; Tanner *et al.*, 1998a; Tanner *et al.*, 2006b; Tran *et al.*, 2001) *T. forsythia* was also found at higher levels in older patients and was frequently related with *P. gingivalis*. Interestingly, *P. gingivalis* was rarely found in the absence of *T. forsythia* (Yang *et al.*, 2004).

Sites with BOP, showed elevated levels of *T. forsythia* proposing a relationship between existing disease and active disease sites (Suda *et al.*, 2003). *T. forsythia* also decreased in frequency and numbers after SRD (Cugini *et al.*, 2000; Darby *et al.*, 2005; Darby *et al.*, 2001; Haffajee *et al.*, 1997b; Takamatsu *et al.*, 1999). Antibiotic therapy has also been reported in some studies to suppress *T. forsythia* post therapy (Feres *et al.*, 2001; Rodrigues *et al.*, 2004; Winkel *et al.*, 1997; Winkel *et al.*, 1998).

Some of the virulence factors identified for *T. forsythia* include, a trypsin-like protease; enzymes including sialidase (Ishikura *et al.*, 2003); glycosidase (Hughes *et al.*, 2003); cell surface assisted proteins (Sabet *et al.*, 2003; Sharma *et al.*, 1998); extracellular proteolytic enzymes and lipopolysaccharides (O'Brien-Simpson *et al.*, 2004{Tanner,

1985 #1111; Tanner *et al.*, 1985). *T. forsythia* was commonly detected on and in periodontal pocket epithelium in studies, suggesting that this species has the ability to invade (Dibart *et al.*, 1998). Taubamn *et al.*, in 1992 found that serum antibody to *T. forsythia* was found elevated in a number of patients with periodontitis (Taubman *et al.*, 1992).

A significant increase in the frequency of detection of *T. forsythia* was found in ligature induced periodontitis and peri-implantitis in dogs (Nociti *et al.*, 2001).

1.6.5.3 Orange Complex Bacteria

The orange complex bacteria include *P. intermedia*, *P. micros* and *F. nucleatum*. This complex of bacteria is very closely associated with the red complex with species in the orange complex preceding colonization by the red complex (Socransky *et al.*, 1998).

1.6.5.3.1 *P. intermedia*

P. intermedia, formerly known as *Bacteriodes melaninogenicus subspecies intermedius* is regarded as a periodontal pathogen, although not as strongly associated with periodontitis as the red complex bacteria. This Gram-negative rod-shaped bacterium can be found in anaerobic environments of the gingival crevice as well as the gastrointestinal tract. *P. intermedia* can be found in both healthy and diseased pockets and have been associated with a larger proportion of sites with BOP (Zhou *et al.*, 2014). *P. intermedia* has been found elevated in cases of acute necrotizing ulcerative gingivitis (Loesche *et al.*, 1982) but has also been detected in some forms of periodontitis (Dzink *et al.*, 1983; Moore *et al.*, 1985; Tanner *et al.*, 1979). This bacteria was found to be more prevalent in periodontal subjects than healthy subjects in the Van Winkelhoff *et al.*, (2002) study, however, *P. intermedia* only made up 6% of the total bacterium in both groups (van Winkelhoff *et al.*, 2002). There have been mixed views with regards to *P. intermedia* and disease activity with some studies finding no significant difference between *P. intermedia* levels (Haffajee *et al.*, 1991c; Tanner *et al.*, 1996) while some reporting an increase in *P. intermedia* to be associated with disease activity (Dzink *et al.*, 1988). *P. intermedia* is detected early in the stages of

disease often preceding and co-existing with the late colonizers, i.e. *T. forsythia*, *P. gingivalis* and *T. denticola* (Mombelli *et al.*, 2000; Socransky *et al.*, 1998; Zhou *et al.*, 2014).

Studies have reported a decrease in *P. intermedia* after SRD (Ali *et al.*, 1992; Bragd *et al.*, 1987; Colombo *et al.*, 2005; Renvert *et al.*, 1990; Socransky *et al.*, 1993; van Winkelhoff *et al.*, 1988; Wikström *et al.*, 1993). A good indicator of periodontal stability is the absence of *P. intermedia* rather than the presence which can indicate progressive bone loss (Chaves *et al.*, 2000; Haffajee *et al.*, 1997b). Research has shown a greater reduction of *P. intermedia* in subgingival plaque samples of patients receiving antibiotics such as amoxicillin and metronidazole than in placebo groups (Rooney *et al.*, 2002).

P. intermedia has a number of virulence properties exhibited by *P. gingivalis*. In-vitro studies have demonstrated the ability of this species to invade oral epithelial cells (Dorn *et al.*, 1998). Only some subjects with refractory periodontitis have displayed elevated serum antibodies to *P. intermedia* (Haffajee *et al.*, 1988b). It was also shown to cause mixed infections on injection in laboratory animals (Hafstrom *et al.*, 1997).

1.6.5.3.2 *P. micros*

P. micros is an anaerobic, Gram-positive, asaccharolytic coccus (Haffajee *et al.*, 1994). It is thought to be a commensal of the oral cavity as it comprises less than 3% of the subgingival flora in periodontally healthy adults (Moore *et al.*, 1982). This pathogen has been linked to mixed anaerobic infections in the oral cavity. However, it has been found more often and in increased percentages in subjects with periodontitis (Moore *et al.*, 1983; Moore *et al.*, 1982; Nonnenmacher *et al.*, 2001; Tanner *et al.*, 1989; Tanner *et al.*, 1979; Yasui, 1989). *P. micros* has also been isolated in active lesions (Dzink *et al.*, 1988; Haffajee *et al.*, 1994; Moore *et al.*, 1991; Rams *et al.*, 1992). Colombo *et al.*, in 2009, found a significantly higher frequency of *P. micros* in patients with refractory periodontitis than in those with treatable periodontitis and with periodontal health (Colombo *et al.*, 2009). van Winkelhoff *et al.*, (2001) found higher

mean levels of *P. micros* in current smokers as compared to non-smokers (van Winkelhoff *et al.*, 2001).

Decreases in levels and frequency of *P. micros* were observed after periodontal treatment in the study by Haffajee *et al.*, in 1988 (Haffajee *et al.*, 1988a). Ciurescu *et al.*, in 2019, found a significant reduction in levels of *P. micros* following the use of lasers with non-surgical therapy six months post-therapy (Ciurescu *et al.*, 2019). Reductions in *P. micros* were also reported by Haffajee *et al.*, (2004) in subjects with refractory periodontitis that were treated with SRD and locally delivered tetracycline, amoxicillin and weekly supragingival plaque removal for three months (Haffajee *et al.*, 2004).

Elevated antibody levels to *P. micros* were found in patients with severe generalised periodontitis as compared to healthy patients or patients with localized juvenile periodontitis (Tew *et al.*, 1985b). *P. micros* produces proteases (Grenier *et al.*, 2006) and has been found to utilize glutathione in hydrogen sulphite formation (Carlsson *et al.*, 1993; Persson *et al.*, 1990). This species in combination with either *P. intermedia* or *P. nigrescens* has been found to cause transmissible abscesses in studies involving mice skin models (van Dalen *et al.*, 1998).

1.6.5.3.3 *F. nucleatum*

F. nucleatum is a Gram-negative anaerobic, spindle-shaped rod that belongs to the *Bacteroidaceae* family (Signat *et al.*, 2011). It is regarded as an important bacterium in the development of dental plaque biofilms and is one of the first Gram negative species to become established in dental plaque biofilms (Signat *et al.*, 2011). This species is a commonly found in subgingival plaque samples of patients with adult periodontitis (Haffajee *et al.*, 1988c; Tanner *et al.*, 1989) and comprises 7-10% of total isolates from different clinical conditions (Dzink *et al.*, 1988; Dzink *et al.*, 1985; Moore *et al.*, 1985). Studies have reported that *F. nucleatum* was found in 80-100% of patients with adult periodontitis (Mombelli *et al.*, 1995; Papapanou *et al.*, 1993; Savitt *et al.*, 1984; Wikström *et al.*, 1993). One hundred percent (100%) of the periodontal pockets sampled in periodontal disease subjects in the study by Choi *et al.*, in 2000 harboured *F. nucleatum* whereas 58% of healthy subjects harboured this species (Choi *et al.*,

2000). Ramberg *et al.*, (2003) found patients that abstained from plaque control for 4 days had significant increases in levels of *F. nucleatum*, whereas other species such as *P. gingivalis*, *T. forsythia* and *T. denticola* showed no increase in levels (Ramberg *et al.*, 2003). Moore and Moore (1994) found that *F. nucleatum* increased significantly in sites with mild gingival inflammation as compared to sites with no inflammation. *F. nucleatum* was also found to maintain its proportions as gingivitis progressed and periodontitis developed (Moore *et al.*, 1994).

F. nucleatum has been isolated from active sites (Dzink *et al.*, 1988; Moore *et al.*, 1991; Tanner *et al.*, 1987b), periodontal abscesses (Herrera *et al.*, 2000) and has also been correlated with pocket depths (Dzink *et al.*, 1985).

Decreases in *F. nucleatum* after successful therapy has been reported in some studies (Haffajee *et al.*, 2006b; Van der Velden *et al.*, 2003). However, some studies have reported increases in this species following SRD (Colombo *et al.*, 2005; Haffajee *et al.*, 1997b; Wikström *et al.*, 1993).

Earlier studies showed that *P. gingivalis* co-aggregated well with *F. nucleatum* in an aerated environment and *F. nucleatum* facilitated the growth of *P. gingivalis* (Bradshaw *et al.*, 1998). This was demonstrated again by Diaz *et al.*, (2002) where *F. nucleatum* was shown to support the growth of *P. gingivalis* in an environment that was aerated and depleted of carbon dioxide. This study proposed that *F. nucleatum* may have an indirect role in plaque maturation by aiding in the survival of *P. gingivalis* and other anaerobes less tolerant to oxygen. In addition, *F. nucleatum* may create an environment with more carbon dioxide necessary for the growth of *P. gingivalis* (Diaz *et al.*, 2002). Sharma *et al.*, (2005) demonstrated a synergistic relationship between *F. nucleatum* and *T. forsythia* (Sharma *et al.*, 2005).

Subjects with periodontitis had higher serum antibody titres (Immunoglobulin A and Immunoglobulin M titres) serum against the lipopolysaccharides of *F. nucleatum* as compared to healthy individuals (Onoue *et al.*, 2003). *F. nucleatum* possesses numerous virulence factors that allow this species to survive in hostile environments by modulating the host's immune response (de Andrade *et al.*, 2019). Some of the virulence factors include adhesions (Han, 2015), endotoxins (lipopolysaccharides) (Hase *et al.*, 1977; Hofstad *et al.*, 1979), serine proteases (Bachrach *et al.*, 2004) and outer membrane proteins (Kaplan *et al.*, 2010). *F. nucleatum* was found more

pathogenic in mixed cultures with *P. gingivalis* or *P. intermedia* than pure cultures in mouse model studies (Baumgartner *et al.*, 1992). This species was also found to be cause destruction of the periodontium in studies involving gnotobiotic rats (Irving *et al.*, 1975).

1.6.5.4 Orange-associated Complex

The role of members of the orange-associated complex including *C. rectus* and *E. nodatum* in causing periodontal disease has been less clear as compared to the pathogens in the red and orange complex (Haffajee *et al.*, 1994). They appear immediately after the early-colonizers and have low pathogenicity except for *E. nodatum* (Socransky *et al.*, 1998).

1.6.5.4.1 *C. rectus*

C. rectus can be described as an anaerobic, Gram negative, short, motile vibrio which uses hydrogen or formate as its energy source (Haffajee *et al.*, 1994). It is widely distributed in primary, mixed and adult dentitions and has been detected in plaque samples of healthy and subjects with periodontal disease (Gmür *et al.*, 1994). *C. rectus* has been detected in higher numbers in diseased sites as compared to healthy sites (Lai *et al.*, 1992; Lippke *et al.*, 1991; Moore *et al.*, 1985; Moore *et al.*, 1983). This pathogen was found in combination with other pathogenic bacteria in refractory periodontitis (Haffajee *et al.*, 1988b). It has also been found more frequently in sites displaying active periodontal destruction (Dzink *et al.*, 1988; Dzink *et al.*, 1985; Rams *et al.*, 1993; Tanner *et al.*, 1989).

After successful periodontal therapy, *C. rectus* was found to have reduced in numbers and was detected less frequently (Haffajee *et al.*, 1988a; Tanner *et al.*, 1987a). The association of *C. rectus* with periodontal disease progression is not as strong as the pathogens described above, possibly due to it being an opportunistic pathogen with its colonization compatible with periodontal health (Gmür *et al.*, 1994). *C. rectus* correlated with active periodontal sites and resulted in elevated systemic antibody responses (Ebersole *et al.*, 1987). This bacteria produces a leukotoxin and was found

to elevate local immune response and promote inflammation of the periodontal tissues by stimulating human gingival fibroblasts to secrete interleukin- 6 (IL-6) and interleukin-8 (IL-8) (Dongari-Bagtzoglou *et al.*, 1996).

1.6.5.4.2 *E. nodatum*

E. nodatum is a Gram positive, anaerobic pleomorphic rod-shaped periodontal pathogen which has been recently added to the red complex bacteria (Haffajee *et al.*, 2008a). Using species-specific oligonucleotide probes, *E. nodatum* was found at higher levels in deep rather than shallow pockets (Booth *et al.*, 2004). DNA-DNA checkerboard analysis also found higher numbers of *E. nodatum* in subjects with periodontitis and other studies found a positive correlation between *E. nodatum* and pocket depth and clinical attachment levels (Colombo *et al.*, 2002; Papapanou *et al.*, 2000). Haffajee *et al.*, (2006) using DNA-DNA checkerboard analysis examined subgingival plaque samples from 21,832 periodontal sites in 635 periodontal subjects and 189 periodontal healthy subjects. This study found that *E. nodatum* was strongly associated with chronic periodontitis either in the presence of high levels of *P. gingivalis* and *T. forsythia* but also in subjects where these species were found in lower proportions (Haffajee *et al.*, 2006a). Heller *et al.*, (2012) found that *E. nodatum* was strongly related to generalised aggressive periodontitis (Heller *et al.*, 2012). Similarly, Elabdeen *et al.*, also found elevated levels of *E. nodatum* in a group of Sudanese individuals with aggressive periodontitis (Elabdeen *et al.*, 2015). The percentages of sites colonized by *E. nodatum* was also reported to be higher in smokers than non-smokers (Haffajee *et al.*, 2001). Only slight decreases in *E. nodatum* following SRD were reported in the Haffajee *et al.*, (1997) study (Haffajee *et al.*, 1997b).

Studies have found that *E. nodatum* produced elevated serum antibody responses in subjects with various forms of destructive periodontitis (Tew *et al.*, 1985a; Tew *et al.*, 1985b). Virulence factors include esterases, acid phosphatases and aminopeptidases (Wade, 1996).

1.6.5.5 Green Complex

The green complex includes *E. corrodens* and *Capnocytophaga* sp.(*sputigena*; *gingivalis*; *ochracea*) and are considered early colonisers (Socransky *et al.*, 1998).

1.6.5.5.1 *E. corrodens*

E. corrodens is a Gram negative capnophilic asaccharolytic rod. This pathogen has been detected in both healthy sites (Chen *et al.*, 1992) and in plaque samples of patients with adult periodontitis (Kornman *et al.*, 1991; Papapanou *et al.*, 1993; Savitt *et al.*, 1984; Wikström *et al.*, 1993). *E. corrodens* was found more often in sites of periodontal destruction as compared to healthy sites (Savitt *et al.*, 1984).

There were no significant difference in the distribution of *E. corrodens* between active and inactive sites in the study by Dzink *et al.*, in 1985 (Dzink *et al.*, 1985). However in some studies, *E. corrodens* has been linked to poorly responding sites after periodontal treatment (Haffajee *et al.*, 1988b) and active sites (Ebersole *et al.*, 1987; Tanner *et al.*, 1987a). Some studies have reported that *E. corrodens* was found together with *A. actinomycetemcomitans* in areas of localised juvenile periodontitis (Mandell, 1984; Mandell *et al.*, 1987; Savitt *et al.*, 1984). The distribution of *E. corrodens* was found to be much higher in young patients as compared to older patients. Therefore it could be suggested that this bacteria could be linked to young patients with advanced forms of periodontitis (Suda *et al.*, 2002). Consequently, more research into *E. corrodens* as a probable periodontal pathogen should be considered.

There have been mixed reports regarding the reduction of *E. corrodens* following SRD with Haffajee *et al.*, in 1997 reporting minimal change to *E. corrodens* (Haffajee *et al.*, 1997b). Wikström *et al.*, (1993) found that *E. corrodens* increased significantly after SRD and this increase correlated with PPDs (Wikström *et al.*, 1993).

The virulence factors of *E. corrodens* include lipopolysaccharides, outer membrane proteins, adhesins and exopolysaccharide layer (Chen *et al.*, 1989). The study by Yumoto *et al.*, in 1999 demonstrated that *E. corrodens* prompted the epithelial cells to secrete proinflammatory cytokines (Yumoto *et al.*, 1999). Animal studies have shown

progressive periodontal destruction in rats mono-infected with *E. corrodens* (Behling *et al.*, 1981; Irving *et al.*, 1975).

1.6.5.5.2 *Capnocytophaga* Sp. (*C. sputigena*; *gingivalis*; *ochracea*)

These species are Gram-negative, fusiform bacteria and have been detected in both health and disease (Dahlen *et al.*, 1992a; Papapanou *et al.*, 1993). In some studies, the *Capnocytophaga* sp. was found higher in health (Dzink *et al.*, 1985; Grossi *et al.*, 1994; Haffajee *et al.*, 1991c). *C. sputigena* has been linked with localized early-onset periodontitis (Han *et al.*, 1991; Lopez *et al.*, 1995; Murayama *et al.*, 1982; Savitt *et al.*, 1984; Wang, 1991). Elevated proportions of *C. gingivalis* and other species were found in active periodontal lesions and in some inactive sites in the study by Dzink *et al.*, (1985) suggesting that these species may or may not have a role to play in disease or they may have competing virulent or non-virulent strains (Dzink *et al.*, 1985). *C. ochracea* was found in high frequency in refractory patients (Kornman *et al.*, 1991) whereas Haffajee *et al.*, (1991) study found no relationship between *C. ochracea* and risk of disease progression (Haffajee *et al.*, 1991c).

Mombelli *et al.*, in 1995 reported that this species was still detected following SRD or surgery (Mombelli *et al.*, 1995), whereas some studies reported no changes in levels of this species (Haffajee *et al.*, 1997b; Wikström *et al.*, 1993). Ali *et al.*, in 1992 found that the *Capnocytophaga* sp. decreased after SRD (Ali *et al.*, 1992).

Teles *et al.*, in 2012 compared subgingival and supragingival bacterial species in both periodontally healthy and periodontitis subjects after professional debridement. This study found that in periodontally healthy individuals, the mean proportions of *C. ochracea* and *C. sputigena* showed a significant increase at day 4 in supragingival plaque samples. In both healthy and periodontitis groups, *C. gingivalis* increased significantly at day 2 and 4, which coincided with increase in plaque biomass formation. Days 4-7 showed increases in mean proportions of *C. showae* and *C. ochracea* in both groups. *C. sputigena* increased in the healthy group but not the periodontitis group. Analysis of the subgingival samples in the periodontitis group showed increases in proportions of *C. gingivalis* from days 2-4, whereas *C. sputigena* increased in both healthy and periodontitis groups at day 7 (Teles *et al.*, 2012).

The uneven distribution of virulence factors among isolates for this species is suggestive that pathogenic potential may be linked with only some of the species (Irving *et al.*, 1975; Laughon *et al.*, 1982). Studies have shown that *C. gingivalis* has a stronger proteolytic activity as compared to *C. ochracea* and *C. sputigena* (Gazi *et al.*, 1997). Lipopolysaccharides from *C. gingivalis* has been found to have hemagglutinating activity in contrast to lipopolysaccharides from *C. ochracea* (Okuda *et al.*, 1987).

1.6.6 Effect of Treatment on Periodontal Pathogens

Although clinical studies have found that a good clinical response to treatment can result in a reduction of periodontal pathogens, the recolonization of subgingival microbiota following periodontal therapy is a common occurrence. Repopulation of subgingival sites is especially common in deeper pockets, concavities and areas associated with intraosseous defects as these sites may be difficult to debride (Ishikawa *et al.*, 2004). Periodontal pathogens have also been found in dentinal tubules which cannot be accessed with conventional therapy (Umeda *et al.*, 2004).

A number of clinical trials have shown that periodontal pathogens including *T. forsythia*, *P. intermedia*, *P. gingivalis* and *T. denticola* decrease in levels and prevalence following SRD (Darby *et al.*, 2001; Haffajee *et al.*, 1997b; Takamatsu *et al.*, 1999). Renvert *et al.*, (1990) found that *P. gingivalis* was eliminated in majority of sites after root debridement, however *A. actinomycetemcomitans* was still found in high proportions. This could be a result of *A. actinomycetemcomitans* being able to invade tissues and the difficulty in removing this bacteria non-surgically (Renvert *et al.*, 1990).

A decrease in *P. gingivalis* has been associated with improvement in clinical outcomes, with this bacteria also being detected at high levels at sites that respond poorly to therapy (Renvert *et al.*, 1990 ; Wikström *et al.*, 1993). Even though *T. forsythia* decreased following non-surgical therapy, this bacterium was found to be more difficult to eradicate. *T. forsythia* decreased from 43% to 27% after non-surgical therapy in the Haffajee *et al.*, study (Haffajee *et al.*, 1997a). Takamatsu reported a decrease from

77.9% to 35.6% (Takamatsu *et al.*, 1999) whereas Darby *et al.*, reported a decrease from 57.6% to 36.36% on a site level (Darby *et al.*, 2001).

The study by Haffajee *et al.*, (1997) on 57 subjects recorded both clinical and microbiological data before the start of treatment and repeated records at three, six and twelve months. This study reported that although *P. gingivalis* and *B. forsythus* (*T. forsythia*) decreased significantly post-therapy there were no further decreases at six and nine months. Post-SRD, most sites in most subjects were recolonized by the species evaluated, emphasizing the importance of regular periodic maintenance (Haffajee *et al.*, 1997b). Cugini *et al.*, (2000) also reinforced the importance of SPT in decreasing the counts of periodontal pathogens. Subjects were seen on a three-monthly basis for hygiene maintenance and OHI. Significant decreases in proportions, prevalence and levels post therapy for suspected periodontal pathogens including *B. forsythus* (*T. forsythia*), *P. gingivalis*, *T. denticola* and *S. constellatus* were reported in this study. The most significant reductions of suspected pathogens were seen three months post therapy with some species reducing even further at twelve months (Cugini *et al.*, 2000).

Doungudomdacha *et al.*, (2001) included 541 sites in 50 adult periodontitis subjects. Using qualitative real-time PCR method (q-PCR), this study related the numbers of *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* to clinical parameters at diseased and healthy periodontal sites before and after non-surgical periodontal therapy. Treatment was provided at the three-month recall in non-responding sites. A statistically significant decrease in counts of all three bacterial species was seen two weeks post treatment. However, in untreated areas, there were fluctuations in the number of bacteria in the first week of sampling. *P. gingivalis* was associated with pocket depth ($p=0.006$) and AL ($p=0.010$). However, both *P. intermedia* and *A. actinomycetemcomitans* were not associated with clinical parameters. At 2-8 weeks, *P. gingivalis* was higher in posterior sites than anterior sites and was found three times more in sites greater than 3 mm than in sites less than 3 mm, suggestive that a threshold number of *P. gingivalis* is associated with early signs of disease. The threshold number of cells were reported as 2.6×10^4 for *P. gingivalis* in this study; however, whether this number of cells was predictive of disease progression could not be determined in this study. Although proportions of *P. gingivalis*, *P. intermedia* and *A.*

actinomycetemcomitans decreased (86–99%), none of the bacteria were completely eliminated, suggesting that organisms re-establish themselves (Doungudomdacha *et al.*, 2001).

In an attempt to evaluate the value of clinical and microbiological parameters to predict treatment outcomes, Brochut *et al.*, (2005) compared clinical and microbiological data at baseline and 6 months after completion of non-surgical periodontal therapy. Site-specific data proved difficult in predicting clinical and microbiological outcomes. However patient specific data showed that subjects with good clinical outcome had no detectable levels of *A. actinomycetemcomitans* at month six, *P. gingivalis* was eliminated in two subjects and only two subjects were positive for *P. gingivalis* in isolated sites. However, subjects in the poor response group were still positive for *A. actinomycetemcomitans* and for *P. gingivalis*, *T. forsythia* and *T. denticola* throughout the study period (Brochut *et al.*, 2005).

Having information on sites at risk of disease progression will be valuable to clinicians, as this information can help modify periodontal treatment for individuals. The longitudinal study by Byrne *et al.*, (2009) monitored disease progression in patients included in an SPT program. Proportions of *P. gingivalis*, *T. denticola*, *T. forsythia*, *F. nucleatum*, and *P. intermedia* were determined using real-time PCR methods. Clinical parameters were used to predict disease progression during a 12-month period. Disease progression was indicated by at least 2 mm of clinical attachment loss. Twenty-five sites of the 205 sites experienced disease progression. Microbiological analyses of 108 baseline subgingival plaque samples revealed that *P. gingivalis* was only found in the presence of *T. forsythia* and *T. denticola*. However, *T. denticola* and *T. forsythia* were frequently found in the absence of *P. gingivalis*. This finding is suggestive that *T. denticola* and *T. forsythia* may provide an environment to facilitate the colonization and proliferation of *P. gingivalis*. Results of this study found significant partial correlations in sites undergoing intermittent periodontal disease progression between *P. gingivalis* and *T. forsythia* ($r=0.55$, $p<0.001$) and *T. denticola* and *T. forsythia* ($r = 0.43$, $p = 0.04$). It was also found that an interaction between *P. gingivalis* and *T. denticola* increased the probability of a site undergoing imminent disease progression in this population. Monitoring proportions of *P. gingivalis* and *T. denticola*

could possibly help identify sites at significant risk of disease progression which could potentially assist with treatment (Byrne *et al.*, 2009).

Charalampakis *et al.*, (2013) in a two-year longitudinal study aimed to identify sites at risk for further progression in the future. Clinical and microbiological data were obtained at baseline (in 2003) from 50 patients with chronic periodontitis. Patients completed non-surgical periodontal therapy and were referred to their general dentist for ongoing periodontal maintenance therapy and were re-examined in 2006 and 2008. Of the 100 sites examined at baseline, 62 of these sites were examined in the f/up appointments. Microbiological sampling using DNA-DNA hybridization techniques involving 25 bacterial species, analysed two sites, one healthy site and one with periodontitis. Results of microbiological testing revealed that *T. forsythia* as well as *F. nucleatum* and *Porphyromonas endodontalis* (non 'red complex bacteria) were detected in four out of the five sites with disease progression. Interestingly, other species, such as *Parvimonas micra*, *E. corrodens*, *P. nigrescens*, *Streptococcus intermedius*, and *Dialister pneumosintes* were also elevated in progressing sites. This study found that quantified bacterial markers showing bacterial load, clinical markers such as BOP and pocket depths could predict future disease progression (Charalampakis *et al.*, 2013).

1.6.7 Techniques Used for Microbial Identification

A number of different methodologies have been developed over the years for microbial identification. Problems with isolation techniques, obtaining exact "uncontaminated" samples in the right location, difficulties with taxonomy, identification and misclassification of bacteria have been identified as difficulties associated with microbial identification. Complications also arose in defining periodontal pathogens, as some may result from, than cause disease. The episodic nature of periodontal disease including disease activity; mixed infections and location of pathogens in healthy sites have also made analysis challenging (Socransky *et al.*, 1992; Socransky *et al.*, 1987).

Developments in molecular biology have resulted in new and advanced detection methods allowing not only to identify single microorganisms but also potential pathogenic microorganisms that exist in communities. The complex subgingival microbiota have many uncultured phylotypes which could possibly be detected by a combination of culture based and molecular techniques (Sanz *et al.*, 2011).

1.6.7.1 Light and Electron Microscopy

Light microscopy was the first piece of apparatus that was used by clinicians to detect microorganisms. Dark field and phase contrast microscopy provided researchers with information on shape, size and motility of bacteria. Studies by Listgarten and Hellden in 1978 revealed diseased sites harbour larger numbers of motile rods, spirochetes and a reduction in coccoid forms. Spirochetes which are strongly associated with disease were easily detected using this technique (Listgarten *et al.*, 1978; Suchett-Kaye *et al.*, 2001).

Electronic microscopic techniques provided greater resolution and were able to offer more detail on cell structure and arrangement of microbial cell appendages (Socransky *et al.*, 2005). Chairside microscopy was also considered to be faster, less expensive and required less equipment and personnel. However, the drawback of this technique was that it lacked sensitivity such as the inability to study specific microbes, determine which species are involved in tissue destruction or determine susceptibility to antibiotic therapies (Greenstein *et al.*, 1985; Suchett-Kaye *et al.*, 2001; Teles *et al.*, 2013). Information from microscopy was also not sufficient to predict recurrence of disease in long term maintenance patients (Listgarten *et al.*, 1989; Suchett-Kaye *et al.*, 2001).

1.6.7.2 Culture-Based Techniques

Culture-based techniques involves collection of plaque samples, using an appropriate medium of transport to transfer to a laboratory, then using selective or nonselective media to disperse and grow aerobic and anaerobic bacterial species (Suchett-Kaye *et al.*, 2001). Bacterial culturing over the years has provided valuable information on aetiology and pathogenesis of periodontal disease. Tanner *et al.*, (1979) used

dominant cultivable methods to identify bacteria in the apical region of advancing periodontal lesions. Gram negative cocci and members of the *Veillonella* group were found in the apical area and this study also correlated certain bacteria with certain clinical symptoms. These findings called for further investigation of bacteria (Tanner *et al.*, 1979). Dzink *et al.*, (1988) found that *Wolinella recta* (*W. recta*), *B. intermedius*, *F. nucleatum*, *Bacteroides gingivalis*, *B. forsythus* were higher in active destructive periodontal sites and the probability of a site being regarded as active, was more likely if certain bacteria such as *B. forsythus*, *B. gingivalis*, *P. micros*, *A. actinomycetemcomitans*, *W. recta* and *Bacteroides intermedius* were found at these sites (Dzink *et al.*, 1988).

However, culture-based techniques have disadvantages. They are slow; only a few samples could be collected from each patient and it can be costly (Moore *et al.*, 1994; Tanner *et al.*, 1998a; Teles *et al.*, 2013; Zambon *et al.*, 1995). Another drawback was that not all viable subgingival bacteria are easily cultivable and there is selective growth where no one medium or assortment of media or culture techniques will be able to culture all bacterial species in the periodontal pocket (Suchett-Kaye *et al.*, 2001).

1.6.7.3 Immunological Assays

Immunofluorescence and enzyme-linked immunosorbent assay (ELISA) have been used to recognize and quantify bacterial types found in subgingival plaque samples. Immunological testing is less time consuming and more sensitive as compared to culture techniques. Immunofluorescent microscopy also has the advantage of reducing the false-positives due to cross-sensitivity (Suchett-Kaye *et al.*, 2001). Immunological assays can provide information on the absolute number of bacteria in the periodontal pocket, however the relative proportion of the total bacteria in the pocket is unknown and this information provides limited value to the clinicians. The specificity of these tests are also a major drawback (Listgarten, 1992).

Bacterial concentration fluorescence immunoassays (BCFIAs) and immunofluorescent microscopy were used to detect periodontal pathogens including *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia* and *F. nucleatum* (Suchett-Kaye *et al.*, 2001).

1.6.7.4 Bacterial Enzyme Tests

Bacterial enzymatic profiles can be used to identify periodontal pathogens. Gram-negative anaerobes including *P. gingivalis*, *T. denticola*, *T. forsythia* and *Capnocytophaga sp.* possess significant amounts of the unique enzyme capable of hydrolysing the synthetic substrate, N-benzoyl-DL-arginine-2-naphthylamide (BANA) (Andrade *et al.*, 2010; Teles *et al.*, 2013). The existence of these organisms in a subgingival plaque samples can be confirmed by the ability of the plaque to hydrolyze BANA (Loesche *et al.*, 1990; Loesche *et al.*, 1992).

SK-013 is an enzymatic method used for detection of peptidase activity in subgingival plaque. This method measures the hydrolysis of N-carbobenzoxymethyl-L-arginine-beta naphthylamide. *P. gingivalis*, *T. denticola*, *B. forsythus* and some strains of the *Capnocytophaga sp.* have been found to produce this peptidase activity (Seida *et al.*, 1992).

A protease based diagnostic method for detection of *P. gingivalis* in gingival crevicular fluid has also been developed. Five *P. gingivalis* specific substrates have been identified by screening the proteolytic peptide substrates and fluorogenic peptides including D-amino acids. Screening of saliva and GCF of patients with periodontitis and healthy patients demonstrated the ability of the substrate to identify the presence of *P. gingivalis* proteases (Kaman *et al.*, 2012).

1.6.7.5 Nucleic Acid Probes

There have been numerous developments in molecular biology that have improved microbial detection methods. Nucleic acid probes, either DNA or RNA, have been beneficial in the identification of periodontal pathogens. Clinical studies have used genomic, cloned and oligonucleotide DNA probes to detect periodontal pathogens. DNA techniques are quicker; require fewer personnel and the results are usually available in a few hours (Papapanou *et al.*, 1997b; Riggio *et al.*, 1996). DNA probes and primers are useful for detection of organisms present at low levels as they do not

rely on cell viability (Papapanou *et al.*, 1997b). They are also more sensitive as compared to cultivation methods (Eick *et al.*, 2011).

Although DNA probes confirm results obtained using culture techniques, an issue with DNA probe technology is that the technique is very sensitive and detects periodontal pathogens in healthy sites and in persons with no disease, making results difficult to interpret and can result in assumptions that patients are at risk (Haffajee *et al.*, 1992; Shiloah *et al.*, 2000). DNA probes can be used with colony lift techniques, which involves bacterial culture, but allows examination of larger number of plaque samples (Suchett-Kaye *et al.*, 2001).

1.6.7.6 The DNA-DNA Checkerboard Hybridization Technique

The DNA-DNA hybridization technique as well as PCR have provided more information on the specific species and were able to provide both the quantitative and qualitative differences associated with gingivitis, destructive periodontal diseases and healthy tissues (Socransky, 1977).

DNA-DNA checkerboard hybridization technique developed by Socransky *et al.*, (1994) has formed a large part of microbiological studies over the last 15 years. This technique permits the simultaneous determination of several bacterial species in either single or multiple plaque samples. Plaque samples are placed into separate Eppendorf tubes and DNA is released from the microorganisms by boiling in NaOH. After neutralization, the DNA is transferred to a single nylon surface membrane which uses the 30 channels of a Minislot device (Socransky *et al.*, 2004). Hybridizations are done in vertical lanes with either digoxigenin-labelled whole genomic probes or 16S rRNA-based oligonucleotide probes incubated with alkaline phosphatase (Socransky *et al.*, 1994b). The advantages of checkerboard hybridization are multiple probes can be used simultaneously to detect DNA, RNA, bacterial or viral samples in larger numbers making it quicker and more efficient. Studies utilizing this technique led to the description of the red and orange complex bacteria associated with periodontal disease destruction (Curtis, 2014).

In 1997, Papapanou *et al.*, used the "checkerboard" DNA-DNA hybridization methodology and compared with culture techniques for analysis of the composition of subgingival bacteria and analysed the diagnostic performance of each technique. In total, 283 subgingival plaque samples were analysed and comprised the following species: *P. gingivalis*, *P. intermedia/Prevotella nigrescens*, *F. nucleatum*, *C. rectus*, *E. corrodens*, *B. forsythus*, *A. actinomycetemcomitans*, *S. sanguis* and *S. mutans*. A higher prevalence of half of the species tested was found with the checkerboard method as compared to the culture techniques. Higher bacterial counts which were statistically significant were found with the hybridization methodology for majority of the species analysed (Papapanou *et al.*, 1997b).

However, there were a few criticisms. Only 40 probes were employed, and this was too low in comparison to the more than 300 bacterial species that colonized the periodontal pocket. Cross reactions between species were common using whole-genomic probes as many species had common regions of DNA and were closely associated. There were also concerns with low sensitivity and not all strains would not be identified. Species that are uncultured and difficult to grow could not be detected (Papapanou *et al.*, 1997b).

1.6.7.7 Polymerase Chain Reaction

PCR is considered a fast and sensitive method for detection of bacterial DNA sequences. PCR methods replicate fragments of DNA or RNA and can theoretically detect a single bacterial cell (Suchett-Kaye *et al.*, 2001; Takamatsu *et al.*, 1999). This method allows detection of pathogens below normal level of detection by culture methods, immunofluorescence, enzyme-based tests and DNA probes (Ashimoto *et al.*, 1996; Riggio *et al.*, 1996; Suchett-Kaye *et al.*, 2001). Past studies using PCR technology were able to detect single pathogenic species and seven or eight putative periodontal pathogens in the same sample. These methods are able to recognize unculturable bacteria including spirochetes. Specific PCR primers and DNA probes can be designed to distinguish bacteria in both healthy and diseased tissues. PCR methods can also be used to monitor antibiotic resistance during periodontal treatment (Manch-Citron *et al.*, 2000; Suchett-Kaye *et al.*, 2001). Studies using PCR were able to describe

only the percentage of sites/patients colonised with specific bacteria before and after therapy (Beikler *et al.*, 2004; Darby *et al.*, 2005; Ehmke *et al.*, 2005; Flemmig *et al.*, 1998b; Fujise *et al.*, 2002). Some of the disadvantages of PCR as described in the review by Sanz *et al.*, (2004) are that PCR is not quantitative and provides information on the presence and absence of species. This technique is also highly sensitive which may lead to very low detection of bacterial cells (Sanz *et al.*, 2004).

The 16S rRNA genes are thought to be present in every bacterium and this method allows for the detection of bacteria. The 16srRNA method can also be used to link species or sets of species with certain oral diseases. Using these methods, bacterial diversity can be determined in periodontal pockets of healthy individuals (Aas *et al.*, 2005), those with gingivitis (Kroes *et al.*, 1999) and with periodontal disease (Paster *et al.*, 2001; Sakamoto *et al.*, 2005).

Ashimoto in 1996 used the 16srRNA method to identify the prevalence of eight periodontal pathogens, *A. actinomycetemcomitans*, *B. forsythus*, *C. rectus*, *E. corrodens*, *P. gingivalis*, *P. intermedia*, *P. nigrescens* and *T. denticola*. Included in this study were 50 patients with advanced periodontitis, 50 adults with slight gingivitis and 50 paediatric patients with slight gingivitis. When the PCR method was compared to culture techniques in 49 of the 50 advanced periodontitis subjects, the PCR method was more sensitive in detecting bacteria than the culture techniques with PCR negative and culture positive results found in only 6% of subjects. PCR and DNA probe detection methods were more compatible, had less cross-sensitivity and had matching results in 84% of the samples for *B. forsythus* and 70% for *P. gingivalis* (Ashimoto *et al.*, 1996).

It is a highly sensitive and culture independent method; however it can be costly; there are difficulties in extracting DNA from some bacteria and distinguishing bacteria that are closely related (Curtis, 2014; Sanz *et al.*, 2004)..

1.6.7.8 Real Time PCR

The real time PCR method developed by Heid *et al.*, (1996) provided an accurate, easy method to detect oral pathogens rapidly, cost-effectively with no cross reaction (Boutaga *et al.*, 2006; Heid *et al.*, 1996). Real time PCR probes and/or primers detect

both living and dead cells whereas culture methods are based on living cells (Atieh, 2008). These tests were found to be more accurate as compared to culture techniques in some studies but not in all. Jervoe-Storm *et al.*, (2005) compared real time PCR with culture methods for the detection of putative periodontal pathogens. This study found that a sensitivity of 94% and specificity of 84% could be reached for detection of *P. gingivalis* using real time PCR. *P. gingivalis* was also detected 87.2% using real time PCR as compared to 44.9% by culture techniques (Jervoe-Storm *et al.*, 2005). Boutaga *et al.*, (2003), reported only a small difference between detection of *P. gingivalis* between culture and real time PCR (42.9% and 45.5%) (Boutaga *et al.*, 2003).

The meta-analysis conducted by Atieh *et al.*, (2008) included 5 studies and found that real-time PCR was highly accurate in detecting *A. actinomycetemcomitans* and *P. gingivalis* compared to the culture technique (Atieh, 2008). Commercially available tests such as the real-time PCR test “Meridol Perio Diagnostics” (GABA International, Münchenstein, Switzerland) are available (Eick *et al.*, 2011).

1.6.7.9 Quantitative Real-Time PCR

The quantitative real-time PCR (qPCR) using fluorogenic DNA probes was introduced to help overcome problems with other methods such as accurately measuring the number of specific bacteria in a sample. qPCR was used to detect five major periodontal pathogens namely, *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. denticola*, and *T. forsythia*. One hundred and sixty-five saliva samples were taken from a case control population of 84 periodontitis subjects and 81 periodontally healthy subjects. This form of testing is useful for large-scale populations to detect bacteria associated with periodontitis (Hyvarinen *et al.*, 2009).

1.6.7.10 RNA-Oligonucleotide Quantification Technique

In order to detect and quantify uncultivated and unrecognized bacteria found in both health and disease, the highly specific and sensitive ROQT was used (Goncalves *et al.*, 2012; Teles *et al.*, 2011). ROQT uses ribosomal RNA as target molecules which is found more in bacterial cells than DNA and hence increases the sensitivity of the

oligonucleotide probes. The entire individual culture is placed onto the membrane and there is no pooling of samples, amplification or dilution bias. Multiple samples can be examined for levels of multiple species at the same time on a single membrane, making this technique cost effective. This highly sensitive technique was able to consistently detect 10^5 bacterial cells, and 10^4 cells of most species. No cross-reactions were detected of the 96 bacteria tested (Teles *et al.*, 2011).

1.6.7.11 Microarray Hybridization

Human Oral Microbe Identification Microarray (HOMIM) uses microarray hybridization on glass slides. This technique not only allows the examination of larger numbers of samples but also allows the simultaneous detection of about 300 oral bacteria including uncultured taxa (Paster *et al.*, 2006). The study by Colombo *et al.*, (2009) used HOMIM to study the subgingival microbial profiles of patients with refractory periodontitis, severe periodontitis and periodontal healthy subjects. Patients with periodontitis presented with a greater diversity of subgingival bacteria as compared to subjects that were deemed periodontally healthy at baseline. The data also showed that in those subjects with refractory periodontitis, species such as *P. micros*, *T. forsythia*, *Prevotella spp.*, *P. gingivalis*, *E. nodatum*, *Eubacterium spp.*, *Selenomonas spp.*, and *Treponema spp.* were more prevalent as compared to the other two groups (Colombo *et al.*, 2009).

1.6.7.12 Chairside Tests

1.6.7.12.1 Rationale for Chairside Tests

Information on patients at risk is invaluable to the clinician not only at the initial examination but also during the long-term maintenance of patients with periodontal disease. Chairside testing can assist in the clinical management of patients, as it can provide the clinician with immediate results, allowing the commencement of the appropriate treatment. Chairside tests can reduce costs such as shipping/postage

costs, fees for analysis and use of laboratories. However it is important that they are reliable, simple to use and at a low cost (Srivastava *et al.*, 2017).

1.6.7.12.2 The Evalusite Chairside Test

The Evalusite chairside test was produced by the Eastman Kodak company. It used paper points that allowed for the visual detection and differentiation of antigens from *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia*. This test used polyclonal antibodies to counter against *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia*. It was found to be effective for pooled or single samples and there were no problems with cross-reactivity. However, it was able to detect only three of the potential pathogens and the detection level was at a threshold of 10^4 . Evalusite was commercially available for only a short time as the reliability of the test was questioned (Boyer *et al.*, 1996; Ellwood *et al.*, 1997; Suchett-Kaye *et al.*, 2001).

1.6.7.12.3 The Microbial-Enzymatic N-Benzoyl-DL-Arginine-2-Naphthylamide (BANA) Test

The BANA test is a simple, quick chairside test which utilizes a chromophore added to a synthetic peptidase as a substrate (Benzoyl- DL-Arginine-Naphthylamide) and results in a blue colour when the presence of *T. denticola*, *P. gingivalis* and/or *T. forsythia* is at least 10^4 (Loesche *et al.*, 1990). BANA tests have been utilized in many studies to detect the presence of periodontal pathogens in plaque and the extent of anaerobic periodontal infection; however these tests were unable to provide information on which of the three species exist in plaque samples (Loesche *et al.*, 1997; Teles *et al.*, 2013).

The BANA test was used to identify different levels of *T. denticola*, *P. gingivalis* and *T. forsythia* or combinations at initial diagnosis and after completion of periodontal therapy. Subjects were sampled at initial diagnosis, immediately following SRD, at 45 days and 60 days. Both the BANA test and the DNA-DNA hybridization tests identified the red complex bacteria in subgingival plaque; however, the best sensitivity for the BANA test was at initial diagnosis (95.54%). The results of the study are suggestive

that BANA tests are most effective when bacterial levels are high, as in the case of this study which was at initial diagnosis (Andrade *et al.*, 2010; Loesche *et al.*, 1990). The BANA test also displays a good association with the ELISA test for the detection of the 3 BANA-positive periodontal pathogens (Suchett-Kaye *et al.*, 2001).

In 1992 Loesche *et al.*, compared the BANA test in the commercially developed Perioscan format to DNA probes and highly specific antibodies to *T. denticola*, *P. gingivalis* and *T. forsythia*. The accuracies of all three methods ranged from 83-92%, indicating that if the combination of these three bacteria is a potential marker for an anaerobic periodontal infection, then the detection methods used were equally precise in diagnosing this infection (Loesche *et al.*, 1992).

1.6.7.12.4 Affirm DPmicrobial Identification Test

The Affirm DPmicrobial (MicroProbe Co., Bothell,WA,USA) semi-automated chairside test can be used directly on plaque samples without bacterial culture and takes less than 40 minutes to complete. Plaque samples are collected using manufacturer's instructions and are analysed using the Affirm processor. It detects *P. gingivalis*, *A. actinomycetemcomitans*, *B. forsythus*, *P. intermedia* and *T. denticola* in plaque samples. Detection levels are determined by the manufacturer. A visible blue colour indicates positive results (Tanner *et al.*, 1998b).

1.6.7.12.5 Biotechnica Diagnostics DNA Probe Test

Biotechnica Diagnostics, Inc. chairside test can be used directly on plaque samples. Once plaque samples are obtained, they are sent to a specialised laboratory for analysis. This chairside test utilizes radioactive probes developed for *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. denticola*, *E. corrodens*, *F. nucleatum*, and *C. rectus* (Soder *et al.*, 1993).

1.6.7.12.6 Perio2000

Volatile sulphide compounds (VSC) are produced by microorganisms such as *P. gingivalis*, *P. intermedia* and *T. forsythia* as a result of degradation of serum proteins (cysteine and methionine). Increase in VSC can be indicative of subgingival microbial load. This digital chairside test displays the sulphide levels at each site sampled. The tip is hydrated with a sterile solution and is inserted subgingivally. Once a reading is obtained, the tip is washed and used in another site (Srivastava *et al.*, 2017).

1.6.7.12.7 Micro-IDent Tests

The Micro-IDent® test by Hain Lifescience uses the 16SrRNA PCR method. This test originally identified five periodontal pathogens. The micro-IDent plus test identifies an additional six species and 11 putative pathogens namely *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia* and *T. denticola*, *P. micro*, *F. nucleatum*, *C. rectus*, *E. nodatum*, *E. corrodens* and *Capnocytophaga sp.*, respectively can be analysed. This method of testing utilizes two distinct techniques, namely PCR and reverse hybridization with species/genus specific probes. DNA- strips are coated with specific probes (conjugate and amplification control) which are corresponding to the amplified nuclei acid (amplicon). The amplicons are chemically denatured and bind to probes (hybridization). The washing step removes any unspecifically bound DNA. An enzymatic colour reaction occurs and is visible as a specific banding pattern on the DNA-strip (Hain-Diagnostics).

Determining thresholds for pathogens are important as some periodontal pathogens may be found in low numbers in healthy subjects (Lau *et al.*, 2004; Mineoka *et al.*, 2008). The micro-IDent tests uses 10^3 for *A. actinomycetemcomitans* and 10^4 for the other species included in the test systems (Eick *et al.*, 2011).

Eick *et al.*, (2011) compared real-time PCR to the micro-IDent test on 25 patients who received SRP and were monitored at baseline and three, six and twelve months after treatment was completed. In total 300 samples were analysed. The results for the four major pathogens, *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *T. denticola*, correlated significantly. The DNA strip technology clearly analysed the

different periodontal pathogens and was useful for analysis in individual diagnosis and treatment of periodontitis patients and was found suitable for dental practices (Eick et al., 2011).

When PCR commercial based hybridization (micro-IDent and micro-IDent Plus) was compared to conventional anaerobic culture methods, the results of the study demonstrated that the overall agreement for both methods for eleven periodontal pathogens was between 78% and 97%. Both methods were good for detection of *E. nodatum*, *T. forsythia* and *P. gingivalis* (97% versus 92%), average for *Capnocytophaga sp.*, *E. corrodens*, *A. actinomycetemcomitans*, and *P. intermedia* (91% versus 89%) and poor for *F. nucleatum*, *Parvimonas micra* (*Micromonas micros*), and *C. rectus* (86% versus 78%). Difficulties with culture methods may be due to bacteria that are closely related such as *P. intermedia* and *P. nigrescens* as well difficulties in keeping periodontal pathogens viable for culture detection methods. Both micro-IDent and micro-IDent plus tests gave a good overall agreement in identifying the main periodontal pathogens (Urban et al., 2010).

For the purposes of this study the Micro-IDent plus test from Hain Lifesciences was utilized. The test supplied data on quality and quantity of eleven periodonto-pathogenic species and their affiliation to the “bacterial complexes”. Bacterial levels of the following bacteria were included: *A. actinomycetemcomitans*, the red complex bacteria consisting of *P. gingivalis*, *T. forsythia*, and *T. denticola*; the orange complex (*P. intermedia*, *P. micros*, *F. nucleatum/periodonticum*) and the orange associated complex including *C. rectus* and *E. nodatum* as well as the green complex which consists of *E. corrodens* and *Capnocytophaga sp.* (*sputigena*; *gingivalis*; *ochracea*). Pooled samples were taken of five sites with the deepest pockets which indicated the pathogen load independent from their localization. Bacterial testing was completed before commencement of non-surgical periodontal treatment; at the three-month re-evaluation appointment and then at twelve months.

1.7 Periodontal Therapy

Nonsurgical and surgical treatment of periodontal disease has formed the basis for management of periodontal disease. However, over the years, our knowledge of the etiology of periodontal disease, the interrelationship of microbial and host factors and the associated treatment outcomes has vastly improved. A balance between the microbes and host defence is essential to maintain periodontal stability.

Treatment methods including both surgical and non-surgical methods are used to maintain this balance and keep microbial levels low (Van der Weijden *et al.*, 2002). Studies have found that the prevalence and levels of periodontal pathogens including *P. gingivalis*, *T. denticola* and *T. forsythia* reduces significantly after subgingival SRD and this reduction correlates with reduced probing depths (Darby *et al.*, 2005; Haffajee *et al.*, 1997b).

Creating a local environment and microflora that is compatible with health is one of the treatment goals (Mlachkova *et al.*, 2014). However, the maintenance of periodontal health is also dependant on ongoing monitoring, good home care practices and continual patient motivation (Axelsson *et al.*, 2004). Good oral hygiene practices to reduce supragingival plaque together with subgingival debridement has more effects on subgingival microflora as compared to supragingival oral hygiene alone (Beltrami *et al.*, 1987; Kho *et al.*, 1985) more especially in pocket depths less than 5 mm (Dahlen *et al.*, 1992a; McNabb *et al.*, 1992).

The success of periodontal treatment is usually measured by reduction of pocket depths, the maintenance or improvement of clinical attachment levels and decrease in BOP (Heitz-Mayfield, 2005; Lang *et al.*, 1986{Heitz-Mayfield, 2005 #359). After completion of periodontal debridement and together with a strict home care regime, reduction in pocket depths is usually observed after a few weeks. Pocket depth reduction is advantageous as it creates a less conducive environment for accumulation of anaerobic pathogens (Adriaens *et al.*, 2004).

1.7.1 Non-surgical Periodontal Therapy

The main aim of non-surgical periodontal therapy is to reduce the living bacteria in the biofilm and remove calcified deposits such as dental calculus from the root surface and subgingival area. SRP has been the “gold standard” for many years. It includes the use of hand instruments, sonic and ultrasonic instruments (Adriaens *et al.*, 2004; Lindhe *et al.*, 2008). SRP with gingival curettage was commonly practiced in the 1970s. However, this was phased out with studies showing that the intentional removal of inner soft tissue wall of pocket did not result in any additional improvement in periodontal health (Echeverria *et al.*, 1983). Non-surgical periodontal debridement was mostly performed using hand instruments in the 1970s and 1980s. This included the removal of contaminated cementum (Daly *et al.*, 1979). However, studies have found that excessive removal of cementum is not necessary as the bacterial endotoxins do not strongly adhere to the root surfaces (Cheetham *et al.*, 1988; Hughes *et al.*, 1986; Moore *et al.*, 1986). This was further supported by the study by Mombelli *et al.*, (1995) that reported that the key element in success of periodontal therapy is reduction of selected periodontal pathogens rather than the removal of contaminated cementum and mineralised deposits during RP (Mombelli *et al.*, 1995).

1.7.2 Changes in Clinical Parameters Following Non-Surgical Periodontal Therapy

After completion of non-surgical periodontal therapy, re-evaluation is essential to compare pocket depths, clinical attachment levels and BOP to baseline data. Even though this data may be limited to a site level and is difficult to use to predict future disease progression, it still provides information of sites with residual pocketing and inflammation which could be at risk of further breakdown (Ishikawa *et al.*, 2004).

1.7.2.1 Probing Pockets Depths and Probing Attachment Levels

Periodontal pockets reduce following mechanical debridement as a result of gain in clinical attachment and gingival recession (Hughes *et al.*, 1978; Proye *et al.*, 1982). Within the first 3 months, the greatest change in probing depth reduction and gain in clinical attachment occurs. However, healing and maturation of the periodontium may only occur over 9-12 months (Badersten *et al.*, 1981; Badersten *et al.*, 1984a; Cugini *et al.*, 2000; Kaldahl *et al.*, 1988; Morrison *et al.*, 1980). It is suggested that re-evaluation of clinical parameters following non-surgical periodontal therapy should not occur before 4 weeks after treatment (Caton *et al.*, 1982; Dahlen *et al.*, 1992a; Kaldahl *et al.*, 1988).

The magnitude of probing reduction and gain in PALs is related to the initial depth of the pocket (Cobb, 1996). The mean reduction in PPDs for PPDs 1-3 mm was 0.03 mm with a mean loss in attachment of 0.34 mm. Mean pocket depth reduction and gains in clinical attachment levels following SRP for PPDs 4-6 mm in non-molar defects were reported as 1.29 mm and 0.5 mm respectively. For pockets depths greater than 7 mm, reduction in mean pocket depths was 2.16 mm and gains in attachment were 1.29 mm (Cobb, 1996; Cobb, 2002; Van der Weijden *et al.*, 2002).

Badersten *et al.*, (1984,1985) conducted a series of studies investigating the effects of non-surgical periodontal therapy on single versus multiple rooted teeth on patients with moderate or advanced periodontal disease and the effects on pocket depth reduction, clinical attachment gain and reduction of clinical inflammation. Sixteen patients with severely advanced periodontitis with pocket depths up to 12 mm were treated non-surgically. Treatment included supragingival and subgingival debridement and plaque control using a split mouth design. Patients were followed every three months over a 24-month period and a total of 852 surfaces including maxillary and mandibular incisors, cuspids and premolars were analysed. This study revealed that sites with initially deep pockets showed more gain in probing attachment. At the start of the study, 305 sites demonstrated pocket depths ≥ 7 mm. This was reduced to 35 sites at the 24-month re-evaluation examination. There was also more gingival recession noted and deeper residual pocketing at 24 months as compared to shallow sites. In addition, pocket depths ≤ 3.5 mm showed loss of probing attachment while pockets ≥ 8 mm

showed a 0.9 – 2.3 mm average gain in attachment. Deeper sites could take up to nine months to respond to therapy, making it difficult to assess these only eight weeks after SRP as maximal healing may not have been achieved (Badersten *et al.*, 1984b).

There are limited clinical studies on the clinical changes occurring in molar furcation sites following non-surgical periodontal therapy. The mean reduction in PPDs for PPDs 1-4 mm was 0.4 mm with a 0.2 mm loss of clinical attachment (Claffey *et al.*, 1990; Kalkwarf *et al.*, 1988). For PPDs 4-6 mm, the mean reduction in PPD ranged from 0.00 mm (Loos *et al.*, 1989) to 1.02 mm (Claffey *et al.*, 1990; Kalkwarf *et al.*, 1988) with mean clinical attachment levels varying from a 0.80 mm loss (Claffey *et al.*, 1990; Loos *et al.*, 1989) to a gain of 0.28 mm (Kalkwarf *et al.*, 1988). For PPDs greater than 7 mm in molar sites, the mean reduction in PPD ranged from 0.00 mm (Loos *et al.*, 1989) and 1.52 mm (Claffey *et al.*, 1990). Clinical attachment levels for these sites varied from a loss of 0.50 mm (Claffey *et al.*, 1990; Nordland *et al.*, 1987) to a gain of 0.84 mm (Kalkwarf *et al.*, 1988). It is evident from these studies that clinical changes in molar sites are less pronounced as compared to non-molar sites.

A systematic review by van der Weijden *et al.*, (2006) addressed the effect of subgingival debridement on BOP, probing depths and attachment levels in patients with chronic periodontitis. Ten studies were included in this review and of the ten, four showed that subgingival debridement was effective in reducing clinical parameters. However, here again initial pocket depth was strongly related to the treatment outcome. In pockets initially greater than or equal to 5 mm, the weighted mean attachment gain was 0.64 mm in patients that received subgingival debridement as compared to supragingival plaque control which was 0.37 mm. Pocket depth reduction was 0.59 mm in patients who received supragingival plaque control as compared to 1.18 mm in patients who received subgingival debridement. Deeper pockets showed more improvements than shallow pockets. This review found that subgingival debridement together with supragingival plaque control is effective in reducing pocket depths and improving clinical attachment levels in patients with chronic periodontitis than supragingival plaque control alone (Van der Weijden *et al.*, 2002). Mlachkova *et al.*, (2014) validated the effectiveness of non-surgical periodontal therapy in pocket depth reduction and clinical attachment gain in patients with moderate chronic periodontitis. The number of pocket depths between 3-5 mm reduced by 34.6% after

therapy. In pocket depths greater than 5 mm, there was a 0.5% reduction in the number of pocket depths within this range with statistical significance not reached. This could be due to the low percentage of deep pockets at baseline. Changes in the number of sites with clinical attachment levels between 3-4 mm were small (1679 sites before treatment; 1879 after treatment) but significant. The number of sites with clinical attachment levels >5 mm was recorded at 919 sites before treatment with only 560 sites observed after treatment ($p < 0.001$) suggestive of coronally positioning of the epithelial attachment or attachment gain (Mlachkova *et al.*, 2014).

1.7.2.2 Gingival Recession

Shrinkage of inflamed marginal gingival tissues can result in gingival recession. Inflammation is reduced by replacement of inflammatory cell infiltrate by collagen rich tissues (Biagini *et al.*, 1988; Caton *et al.*, 1979; Harper *et al.*, 1987; Waerhaug, 1978). Shrinkage of the tissues occurs gradually in an apical direction towards the root surface with the formation of the long junctional epithelium (Caton *et al.*, 1981; Caton *et al.*, 1979). Clinical studies reported the amount of gingival recession recorded for PPDs 1-3 mm in non-molar sites was approximately 1 mm (Badersten *et al.*, 1981; Badersten *et al.*, 1984a, 1984b, 1984c; Badersten *et al.*, 1985a, 1985d, 1987a; Claffey *et al.*, 1990). Mean gingival recession varied between 1.2 mm for PPDs 4-6 mm and 1.9 mm for PPDs greater than 7 mm. The amount of gingival recession was not affected by the type of instrument used, whether it was hand or ultrasonic instruments (Badersten *et al.*, 1981; Badersten *et al.*, 1984a, 1984b; Badersten *et al.*, 1985b). Neither operator variability (Badersten *et al.*, 1985b) or single versus multiple episodes of subgingival debridement affected the amount of gingival recession (Badersten *et al.*, 1984c).

1.7.2.3 Bleeding on Probing

There are different methods to record the presence or amount of BOP. The Ainamo and Bay index is a simple index that can be used to calculate the percentage of sites with BOP (Ainamo *et al.*, 1975). The gingival index (GI) by Loë and Silness assesses the severity and prevalence of gingivitis by examining the quantitative changes. Gingival inflammation is recorded as 0 or absence of inflammation to mild (1),

moderate (2) or severe (3) inflammation (Loë, 1967). The papillary bleeding index described by Saxer and Muhlemann is when a periodontal probe is inserted into the gingival sulcus and bleeding is documented 20-30 seconds after each quadrant has been probed. BOP is scored from 1 (minimal) to 4 (profuse). A percentage is then calculated by adding all bleeding scores and dividing by the number of sites examined (Saxer *et al.*, 1975).

Past studies have reported reduction in BOP following subgingival debridement (Badersten *et al.*, 1981; Badersten *et al.*, 1984b; Cercek *et al.*, 1983; Kalkwarf *et al.*, 1989; Tagge *et al.*, 1975). An average reduction of 50% BOP has been observed in clinical studies in sites with initial probing depths between 4-7 mm (Badersten *et al.*, 1981; Badersten *et al.*, 1984b; Becker *et al.*, 1988; Copulos *et al.*, 1993; Haffajee *et al.*, 1997b; Isidor *et al.*, 1986; Kalkwarf *et al.*, 1989; Lindhe *et al.*, 1987; Pihlstrom *et al.*, 1983; Preshaw *et al.*, 1999; Proye *et al.*, 1982; Renvert *et al.*, 1990).

BOP has been linked to periodontal breakdown in many clinical studies with a higher probability of periodontal AL in sites that repeatedly show BOP at successive SPT appointments (Badersten *et al.*, 1985d, 1990; Claffey *et al.*, 1995; Joss *et al.*, 1994; Lang *et al.*, 1986). However because of the weak correlation between BOP and AL, Lang *et al.*, (1990) suggested that a more reliable predictor of periodontal stability would be the absence of BOP (Lang *et al.*, 1990).

1.7.2.4 Plaque Levels

Compliance with home care procedures plays a major role in long term prognosis following periodontal treatment. Daily plaque control including brushing, flossing and use of interdental aids should be strictly adhered to in patients with periodontal disease. Supragingival plaque levels can be measured using several indices such as the O'Leary plaque index (PI) (O'Leary *et al.*, 1972), the PI of Silness and Loë (Silness *et al.*, 1964), the simplified oral hygiene index of Greene and Vermillion (Greene *et al.*, 1964). The O'Leary PI measures the presence or absence of plaque from four sites of each tooth (O'Leary *et al.*, 1972). However, plaque scores have limited diagnostic value in predicting AL or disease progression (Badersten *et al.*, 1990).

Numerous studies have supported the importance of good supragingival plaque control in the maintenance of periodontal health. Plaque levels especially around the marginal gingiva can result in gingival inflammation. Therefore, meticulous plaque control is emphasized during active periodontal treatment and the supportive periodontal maintenance phase. Studies have found that meticulous plaque control accompanied by professional supragingival cleaning can influence the composition of the subgingival microflora as well as reduce the total number of microorganisms in periodontal pockets (Hellstrom *et al.*, 1996; Katsanoulas *et al.*, 1992). These changes were more evident in pockets <4 mm (Muller *et al.*, 1986; Smulow *et al.*, 1983). However, the depth of the pocket is key, as in the deeper pockets, supragingival plaque control alone was not sufficient to produce changes in the subgingival flora and had no beneficial effect on clinical parameters (Beltrami *et al.*, 1987; Kho *et al.*, 1985{Beltrami, 1987 #424}).

The removal of mature plaque is an essential part of periodontal therapy. There have been many studies conducted in the past examining the removal of supragingival and subgingival plaque and the effect on the ecology of the subgingival bacteria. Subgingival scaling accompanied by supragingival plaque control was found to be more effective in improving periodontal health and has shown to reduce motile subgingival microflora. However, recolonization of subgingival microbiota occurs in patients with poor plaque control. Large numbers of spirochetes and motile rods were seen after 4-8 weeks in the study by Magnusson *et al.*, (1984) suggesting that subgingival debridement followed by proper supragingival plaque control, influences the recolonization of pathogenic bacteria (Magnusson *et al.*, 1984). Ximenez-Fyvie *et al.*, (2000) demonstrated that professional supragingival plaque removal decreased both supra and subgingival bacterial species. However, in this study, it was suggested that factors such as patients' attitude to home care procedures may have changed and this together with regular disruption of the biofilm led to a prolonged effect on the subgingival microbiota (Ximenez-Fyvie *et al.*, 2000c).

However, some studies found there was no significant association with supragingival plaque control and subgingival microbiota (Beltrami *et al.*, 1987; Kho *et al.*, 1985; Lindhe *et al.*, 1983). Mosques *et al.*, (1980) showed that after a single session of SRP with no OHI, there were decreases in proportions of spirochetes in the subgingival flora (Mosques *et al.*, 1980). Similarly, Sordone *et al.*, (1990) found that even two days

after treatment with no OHI, composition of bacteria was similar in healthy sites. However, by 60 days, this changed, and the microflora composition varied from before to after treatment (Sbordone *et al.*, 1990).

1.7.3 Mechanical Versus Manual Instrumentation

The effectiveness of non-surgical methods in treating periodontal disease has been confirmed by numerous studies (Badersten *et al.*, 1981; Badersten *et al.*, 1984b, 1984c; Badersten *et al.*, 1987b). Over the years many modifications to instruments to perform SRD were made to improve calculus removal (Oda *et al.*, 2004). This included the development of curettes with longer shanks for deep subgingival scaling and mini-bladed curettes for the narrow pockets (Landry *et al.*, 1999; Singer *et al.*, 1992).

Powered instruments were developed in the 1950s and 1960s and included magnetostrictive, piezoelectric ultrasonics, and sonic instruments. The advantages of using powered instruments included reduction of operator fatigue, increased efficiency and effectiveness of subgingival debridement with these instruments, less damage to root surfaces and less patient discomfort (Braun *et al.*, 2007; Heitz-Mayfield *et al.*, 2013). Modifications to power-driven scaler tips to include smaller, thinner tips, rounded, diamond coated, and contra-angle inserts have also been developed to improve the efficacy of debridement (Drisko, 1998).

The end result can take longer to achieve when using manual instruments with several studies reporting that sonic or ultrasonic instrumentation can result in 20-50% of time savings when compared to manual instrumentation (Checchi *et al.*, 1988; Copulos *et al.*, 1993; Dragoo, 1992; Drisko, 1998). Badersten *et al.*, in 1981 compared sonic or ultrasonic scaling instrumentation with manual instrumentation in patients with moderate periodontitis (pocket depths 4-7 mm). Clinical improvements were similar in both groups; however, ultrasonic instrumentation was much faster in achieving the desired result (Badersten *et al.*, 1981). Dragoo *et al.*, (1992) tested the efficiency of hand instruments and ultrasonic instruments (modified and unmodified) on 168 root surfaces. The mean operating time per tooth was 9.6 minutes for hand instruments, 8.5 minutes for a modified ultrasonic (slimline), and 6.8 minutes for an unmodified ultrasonic tip to reach a desired result. This study revealed that ultrasonic

instrumentation was more advantageous for use for SRD as compared to hand instruments, as it more efficient and less fatiguing, as compared to hand instrumentation. In addition, the power setting could be increased allowing easier removal of deposits (Dragoo, 1992).

A systematic review by Tunkel *et al.*, (2002) reviewed 27 articles to compare manual versus ultrasonic/sonic debridement. Although the evidence was not strong, no differences were found between the use of ultrasonic/sonic and manual debridement in treatment of chronic periodontitis for single rooted teeth and in multi-rooted teeth. There were also no differences between the two treatment methods in reducing clinical parameters such as PPDs and BOP or gains in clinical attachment levels. However, ultrasonic instrumentation took less time than manual instrumentation (36.6 %), ($p=0.0002$, 95% CI of the standardized effect estimate: 0.39–1.37, heterogeneity $p=0.77$) (Tunkel *et al.*, 2002). D'Ercole *et al.*, (2008) found that both ultrasonic and manual instrumentation were equally effective in terms of reducing clinical and microbiological parameters in deep pockets ≥ 6 mm. However, both manual instrumentation and ultrasonic instrumentation lowered but did not completely eliminate the putative periodontal pathogens (D'Ercole *et al.*, 2006). Studies by Ossterwall *et al.*, (1987) and Copulos *et al.*, (1993) compared manual and ultrasonic scaling and found that microbiological observations were similar for both methods (Copulos *et al.*, 1993; Oosterwaal *et al.*, 1987).

1.7.4 Full Mouth Debridement, Quadrant Debridement and Full Mouth Disinfection

Non-surgical periodontal therapy can include quadrant debridement, full mouth debridement or full mouth disinfection. Full mouth disinfection was developed as a result of the belief that bacteria can recolonize previously treated areas before the completion of debridement by quadrant. It includes full mouth SRD within a 24 hour period, the use of chlorhexidine mouth rinse twice daily, tongue scraping, tonsil spraying with chlorhexidine and subgingival irrigation with chlorhexidine three times within 10 minutes and repeated after 8 days (Quirynen *et al.*, 1995). The pilot study by Vanderkerchove *et al.*, (1996) reported reduction in pocket depths in the full mouth disinfection group especially in deeper pockets as compared to conventional

treatment. However, the sample size used was small (10) (Vandekerckhove *et al.*, 1996). A larger study using a sample size of 24 adult periodontitis and 16 generalised early onset periodontitis and a longer f/up post treatment, supported that full mouth disinfection was better than quadrant SRD (Mongardini *et al.*, 1999). However, a meta-analysis by Eberhard *et al.*, (2015) found no additional benefit between full mouth disinfection and full mouth SRD in the trials included in the analysis. Ten studies comparing full mouth debridement with conventional SRD also did not indicate any advantage in probing depths, clinical attachment levels or BOP. There was no clear evidence from this analysis that full mouth SRD or full mouth disinfection provided any added benefit as compared to conventional SRD. However, there were concerns with comparing the different studies and methodologies; not all studies included full mouth disinfection and therefore definitive conclusions were difficult to make (Eberhard *et al.*, 2015).

A consensus was reached at the Sixth European Workshop on Periodontology, that all three treatment methods including full mouth debridement, staged debridement or full mouth SRD with antiseptics may be recommended for non-surgical treatment of periodontal disease. A review of systematic reviews on periodontal treatment modalities, showed only modest differences between the different treatment modalities suggesting that one treatment method was not superior to the other (Sanz *et al.*, 2008).

1.7.5 Adjuncts to Non-Surgical Periodontal Therapy

1.7.5.1 Lasers

Lasers commonly used in dentistry are carbon dioxide, neodymium:yttrium-aluminum-garnet(Nd:YAG), Erbium:yttrium-aluminum-garnet (Er:YAG), diode and gas lasers (Darby, 2009). In the late 1990s, low powered lasers were used alone or as an adjunct to SRP. These lasers had a photosensitizer or photoactivable agent such as Toluidine Blue O or methylene blue that absorbed light and produced a wavelength to target microorganisms. A systematic review published in 2011 showed only short-term benefits of photodynamic lasers and there was insufficient evidence to support its use

as an alternative to SRD. In addition, no significant microbiological changes were noted; therefore more trials need to be conducted to test the effectiveness of photodynamic therapy as an acceptable treatment option (Heitz-Mayfield *et al.*, 2013; Sgolastra *et al.*, 2013). A systematic review conducted on five randomized controlled trials to evaluate the effectiveness of the use of Erbium-doped: yttrium-aluminium garnet (Er: YAG) laser as compared to SRD in the treatment of chronic periodontitis patients found no significant differences in clinical attachment gain, pocket depth reduction or gingival recession between the two therapies (Heitz-Mayfield *et al.*, 2013; Sgolastra *et al.*, 2013; Sgolastra *et al.*, 2012b). The use of lasers still remains controversial and more research is needed to support the use of lasers in conjunction with non-surgical periodontal therapy (Heitz-Mayfield *et al.*, 2013).

1.7.5.2 Antimicrobials

The use of antimicrobial agents for pocket irrigation has been in use since the late 1970s. Some studies have shown modest improvements in PPDs and only short term benefits of locally delivered antimicrobials used in conjunction with SRD (Rams *et al.*, 1996; Ribeiro *et al.*, 2010; Rosling *et al.*, 1983). In order to obtain bactericidal activity, antimicrobials are needed in very high concentrations. For example, chlorhexidine digluconate at a concentration of 0.5% to 2% needs to be in contact with *P. gingivalis* for 10 minutes and povidone iodine, an antimicrobial agent active against most bacteria, viruses and fungi, must be contact with pathogens for at least 5 minutes at concentrations between 0.5% and 10% (Caufield *et al.*, 1987; Drisko, 2001).

Jose *et al.*, (2016) compared traditional SRD (control) with the use of a chlorhexidine chip, diode laser or the combination of the chlorhexidine chip with the diode laser. Four sites in each of the fifteen patients were randomly selected to receive one of the four treatments. Both test and control sites showed statistically significant reductions in PI, GI, PPDs and clinical attachment levels. At three months, the test groups (chlorhexidine chip only or combination groups, that is chlorhexidine chip and diode laser group) had a statistically significant lower PPD and PAL gain as compared to the control group ($p < 0.05$). However, no significant difference was found between the diode laser group and the control group (Jose *et al.*, 2016).

1.7.5.3 Systemic Antibiotics

Systemic antibiotics used in conjunction with non-surgical debridement for suppressing the persistent periodontal pathogens in deep pockets, concavities, furcation's and within the periodontal tissues has been advocated for many years, but has also remained controversial, with the benefits of systemic antibiotics noted in only select cases. Systemic antibiotics can include the following namely, amoxicillin, tetracycline, doxycycline, metronidazole, clindamycin, amoxicillin + clavulanic acid (augmentin), azithromycin, metronidazole + amoxicillin, spiramycin and minocycline.

A. actinomycetemcomitans which has been linked with more aggressive forms of periodontal disease, can be difficult to eradicate with non-surgical therapy alone. The use of systemic antibiotics can benefit patients with high levels of *A. actinomycetemcomitans*. In addition, it has shown to be beneficial in patients with high levels of *P. gingivalis* and in deep pockets greater than 6 mm (Mombelli *et al.*, 1994a; Mombelli *et al.*, 2000). Improvements in clinical attachments levels were reported with the use of systemic antimicrobials by Haffajee *et al.*, (2008) (Haffajee *et al.*, 2008a).

The systematic review by Haffajee *et al.*, (2003) found that for majority of the comparison studies, systemic antibiotics were beneficial in improving attachment levels. Statistically significant improvements in attachment levels was observed with the use of tetracycline, metronidazole and the combination of amoxicillin plus metronidazole showing borderline statistical significance. Improvements in mean PAL were observed in both chronic and aggressive periodontitis patients with more benefits in the aggressive cases of periodontitis. However, many other factors need to be taken into consideration prior to administration of systemic antibiotics (Haffajee *et al.*, 2003b). Goodson *et al.*, (2012) also reported that the combination of amoxicillin and metronidazole with SRD was found to be beneficial in reduction of probing depths and clinical attachment gain. Improvements in clinical outcomes with use of adjunctive antimicrobials were observed at 24 months (Goodson *et al.*, 2012). More attachment gain and reduction in PPDs were also described in the systematic review by Sgolastra *et al.*, (2012). This meta-analysis found that significantly more attachment gain (0.2 mm) and reduction of PPDs (0.4 mm) was found when SRD was done in combination with amoxicillin and metronidazole (Sgolastra *et al.*, 2012a).

The double masked, placebo-controlled study by Han *et al.*, (2012), investigated the efficacy of SRD plus the use of azithromycin on patients with severe generalised chronic periodontitis. Both groups showed significant improvements in clinical parameters in deep and moderate pockets. However, no additional benefit of using azithromycin over non-surgical periodontal treatment was found. Microbiological analysis showed that azithromycin did not provide any additional effect on periodontal pathogens. Although *F. nucleatum*, *P. gingivalis*, *P. intermedia*, and *T. forsythia* decreased in both groups, none were eradicated, and most bacteria tended to reappear after 6 months except for *P. intermedia* (Han *et al.*, 2012). Similarly Fonseca *et al.*, (2015) found groups treated with azithromycin did not show statistically significant differences in pocket depths, reduction in percentage of periodontal disease sites or lower microbial levels at 90 and 180 days after treatment (Fonseca *et al.*, 2015). However, the presence of putative bacteria does not necessarily show the presence or absence of disease, higher numbers of bacteria are better indicators (Han *et al.*, 2012; Socransky *et al.*, 1992).

In 2008, Sanz *et al.*, reviewed the use of systemic antimicrobials, recommending that systemic antimicrobials should be used for periodontal conditions such as aggressive periodontitis or in severe or progressive forms of the disease. The review also suggested that they are used in combination with non-surgical therapy and that optimum outcomes could be attained if the drug therapeutic levels are reached at the completion of debridement and that debridement should ideally be completed within seven days (Sanz *et al.*, 2008). A systematic review by Garcia *et al.*, (2015), although with several limitations due to the high heterogeneity of clinical studies included in the review, showed some evidence that systemic antibiotics as an adjunct to mechanical debridement may result in improvements in pocket depths and clinical attachment levels as compared to mechanical debridement alone. Here again, this was noted more especially in cases of aggressive periodontal disease and in patients with pockets greater than 6 mm (Garcia *et al.*, 2015).

The prescribing of systemic antimicrobials should be assessed on a case by case basis. Systemic antibiotics should be used with caution, as there can be concerns with antimicrobial resistance, allergic reactions and adverse interactions with other medications. Factors such as patient compliance, adequate debridement, and ongoing

maintenance are imperative when considering the use of systemic antibiotics (Heitz-Mayfield *et al.*, 2013).

1.7.5.4 Guided Pocket Recolonization

Therapies including probiotic and replacement therapies has shown to be beneficial in caries prevention, pharyngitis and otitis media, however the benefits of using beneficial bacteria in periodontal pockets has not been extensively studied. A study by Teughels *et al.*, in 2007 on beagle dogs, showed that when beneficial bacteria were used as an adjunct to SRD in periodontal pockets, it resulted in clinically significant delay and reduction of recolonization of subgingival microbiota as well as reduction in inflammation (Teughels *et al.*, 2007).

1.7.5.5 Use of Host-Modulating Drugs

The concept of host modulation in dentistry was introduced by William and Golub in 1990. Host modulation involves the use of pharmacological agents to modulate the host responses involved in the pathogenesis of periodontal destruction. The main aim is to reduce tissue destruction and stabilise or regenerate the periodontium by modifying or regulating the destructive parts of the host response and increasing the protective or regenerative responses. There are various host modulation agents been researched including non-steroidal anti-inflammatory drugs, tetracyclines, chemically modified tetracycline, anticytokines agents, recombinant tissue inhibitor of matrix metalloproteinase, synthetic matrix metalloproteinase inhibitors and bisphosphonates. (Shinwari *et al.*, 2014).

Systemic sub-antimicrobial dose of doxycycline (Periostat®) has been used in some clinical trials. A summary of several trials using different doses of doxycycline and comparing to placebos were reported by Ashley (1999). It was found that sub-antimicrobial doses of doxycycline used as an adjunct to debridement reduced collagenase levels in both gingival crevicular fluid and gingival biopsies and had a positive impact on clinical attachment levels, probing depths, BOP and alveolar bone

loss (Ashley, 1999). Caton *et al.*, (1999) also reported increase in attachment levels and decrease in probing depths following the use of sub-antimicrobial doses of doxycycline as an adjunct to SRD in patients with adult periodontitis in randomised controlled trials (RCT) of 9 and 12 months duration (Caton, 1999).

A systemic review by Sulijava *et al.*, (2019) investigating the efficacy of host modulation therapy (HMT) agents in periodontitis supports the use of HMT agents in periodontics in *in-vitro*, *in-vivo* and in human studies. HMT targeted specifically on anti-inflammatory and antioxidants can be a good therapeutic approach which can result in clinical benefits. Although it should be used with caution especially in immunocompromised patients (Sulijaya *et al.*, 2019).

Cross sectional data have reported that low doses of acetylsalicylic acid or aspirin have been found to have some benefit in improving periodontal health (Drouganis *et al.*, 2001; Flemmig *et al.*, 1996). Flemmig *et al.*, (1996) found that a combination of conventional therapy and systemically administered acetylsalicylic acid may result in synergistic therapeutic benefits in patients with untreated adult periodontitis. Drouganis *et al.*, (2001) suggested that low doses of aspirin daily may reduce the risk of AL in ex-smokers (Drouganis *et al.*, 2001).

The use of omega-three fatty acids or fish oil supplements for periodontal disease has been researched over the years. There is emerging evidence that periodontitis-related inflammatory markers may be reduced with the combination of non-surgical periodontal debridement and the use of fish oil supplements that contain long-chain omega-3 polyunsaturated fatty acids(LCn3PUFA),omega-3 fat eicosapentaenoic acid (EPA) or Docosahexaenoic acid (DHA) (Chee *et al.*, 2016).

1.7.6 Healing Following Periodontal Therapy

SRD involves the removal of irritants, resulting in reduction of gingival inflammation, accompanied by reduction of the periodontal pocket depths and gains in PALs. Gingival recession can occur due to the reduction of swelling of the marginal gingiva

and gradual shrinkage of the tissues in an apical direction towards the root surface (Adriaens *et al.*, 2004).

Healing can follow different patterns. It can occur by the formation of the long junctional epithelium, connective tissue attachment or even periodontal regeneration (Caton *et al.*, 1980; Listgarten *et al.*, 1979; Polimeni *et al.*, 2006). Histological studies have shown that healing by formation of a long junctional epithelium occurs where a thin epithelium consisting of two or three layers align parallel to the tooth surface and extends apically between the tooth surface and connective tissue (Listgarten *et al.*, 1979; Waerhaug, 1978).

Due to the highly proliferative activity of the long junctional epithelium, it can be produced rapidly during wound healing and can occur within one to two weeks (Waerhaug, 1978). It has been suggested that the long junctional epithelium is not the final healing stage, but rather a transitory feature of the healing process leading to connective tissue attachment. It has also been proposed that at 4 weeks post-therapy the long junctional epithelium can migrate coronally which results in a shorter long junctional epithelium (Shimono *et al.*, 2003).

Connective tissue repair is said to occur when the collagen fibres are oriented parallel or perpendicular to the root surface previously exposed to periodontal disease or previously deprived of its periodontal attachment. Periodontal regeneration on the other hand, is when there is de novo formation of cementum, a functionally oriented periodontal ligament, alveolar bone and gingiva (Polimeni *et al.*, 2006). Horizontal and vertical alveolar bone defects are evident in patients with periodontal disease with alveolar bone regeneration occurring in some cases where vertical defects are present (Lindhe, 1982).

1.7.7 Challenges Faced with Non-Surgical Debridement

Non-surgical periodontal therapy results in reduction of pocket depths and gains in clinical attachment; however, residual calculus can still be found after SRD (Anderson *et al.*, 1996; Brayer *et al.*, 1989; Buchanan *et al.*, 1987; Caffesse *et al.*, 1986; Dragoo, 1992; Johnson *et al.*, 1989; Nagy *et al.*, 1992; Rabbani *et al.*, 1981; Waerhaug, 1978;

Wylam *et al.*, 1993). Issues with visibility, accessibility and anatomical factors including furcation involvement and root concavities are some of the contributing factors (Adriaens *et al.*, 2004; Cobb, 1996; Greenstein, 1992, 2000; Heitz-Mayfield *et al.*, 2013; Lindhe *et al.*, 2008). Single rooted teeth and posterior teeth with intact furcations respond much better to periodontal therapy as compared to multi-rooted teeth with furcation involvement (Kaldahl *et al.*, 1996a). It is also very difficult to predict outcomes especially in teeth with furcation involvement and angular defects. Complete debridement of furcation areas is difficult to achieve non-surgically (Breininger *et al.*, 1987; Heitz-Mayfield, 2005; Lee *et al.*, 1996; Oda *et al.*, 1989; Wylam *et al.*, 1993).

Other factors that influence outcomes of non-surgical periodontal therapy include failure to continue with ongoing SPT and poor adherence to suggested oral hygiene practices (Wilson, 1996a, 1996b). Systemic conditions such as diabetes affect healing of the periodontal tissues (Grossi *et al.*, 1998; Scannapieco, 1998). Smoking is a highly significant risk factor for periodontitis with smokers having a decreased immune response and poor healing following periodontal therapy (Anonymous, 1999; Tonetti, 1998).

The recolonization of the bacteria is not fully understood. It may take several months for bacteria to recolonize the pocket following SRD and good oral hygiene; however, in patients with poor oral hygiene practice, bacteria may re-establish within 40-60 days following debridement. Recolonization of microbiota may occur by 120-140 days in deeper pockets irrespective of multiple subgingival debridement and meticulous oral hygiene practices (Drisko, 2001; Sbordone *et al.*, 1990).

Complete removal of calculus and bacteria is not possible following instrumentation (Anderson *et al.*, 1996; Brayer *et al.*, 1989; Buchanan *et al.*, 1987; Caffesse *et al.*, 1986; Dragoo, 1992; Johnson *et al.*, 1989; Nagy *et al.*, 1992; Rabbani *et al.*, 1981; Waerhaug, 1978; Wylam *et al.*, 1993). Research conducted by Sherman *et al.*, (1990), demonstrated that the thoroughness of subgingival instrumentation is difficult to determine in pockets greater than 4 mm (Sherman *et al.*, 1990). It was reported that, even after 12–15 minutes of treatment per tooth, 63% of root surfaces still harboured residual calculus (Sherman *et al.*, 1990). Similarly a study by Anderson in 1996, found that residual calculus may still not be removed even after repeated instrumentation (Anderson *et al.*, 1996). Even in shallow pockets up to 3 mm, studies have found that

4-43% of calculus remnants are found (Brayer *et al.*, 1989; Buchanan *et al.*, 1987; Clifford *et al.*, 1999; Gellin *et al.*, 1986; Rabbani *et al.*, 1981).

Instrumentation becomes less effective as the pocket depth increases (Kawanami *et al.*, 1988; Rabbani *et al.*, 1981; Sherman *et al.*, 1990; Waerhaug, 1978). Stambaugh *et al.*, (1981) described the inability to remove all subgingival plaque and calculus in pocket depths more than 3.73 mm (Stambaugh *et al.*, 1981). Fifteen to thirty-eight percent of treated sites has residual plaque and calculus in pocket depths 4-6 mm (Brayer *et al.*, 1989; Buchanan *et al.*, 1987; Carey *et al.*, 2001; Clifford *et al.*, 1999; Gellin *et al.*, 1986; Kawanami *et al.*, 1988; Rabbani *et al.*, 1981). These percentages increased for deeper pockets with studies showing that PPDs greater than 6 mm, 19-66% of sites had residual plaque and calculus (Anderson *et al.*, 1996; Brayer *et al.*, 1989; Buchanan *et al.*, 1987; Carey *et al.*, 2001; Clifford *et al.*, 1999; Gellin *et al.*, 1986; Kawanami *et al.*, 1988; Lee *et al.*, 1996; Rabbani *et al.*, 1981).

Historically, RP was used utilizing both hand and powered instruments with the main goal being obtaining a smooth, glassy root surface. However, research has established that calculus does not necessarily cause disease, the cementum does not become significantly infected and bacteria and toxins only loosely adhere to the root surface and can be removed by lighter instrumentation without damage to the root surface (Hughes *et al.*, 1986, 1990; Ito *et al.*, 1985; Moore *et al.*, 1986; Nakib *et al.*, 1982; Nyman *et al.*, 1986). The aggressive approach to remove contaminated cementum has been questioned and removal of excess cementum is not necessary (Adriaens *et al.*, 2004; Heitz-Mayfield *et al.*, 2013; Hughes *et al.*, 1986; Moore *et al.*, 1986; Ohshima, 1987; Smart *et al.*, 1990). Hence the replacement of RP with root surface debridement (Cheetham *et al.*, 1988; Smart *et al.*, 1990).

Reducing PPDs to a manageable depth of 4 mm or below is one of the aims of non-surgical periodontal therapy. These shallow sites can be well debrided (Waerhaug, 1978) and with good oral hygiene practices, can potentially reduce the build-up of periodontal pathogens (Beltrami *et al.*, 1987; McNabb *et al.*, 1992).

1.7.8 Surgical Periodontal Therapy

In areas where there is persistent inflammation, surgical intervention may be required. Commonly used procedures include access or open flap debridement, gingivectomy or apically positioned flap surgery. However, in the 1980s when more was learned about the pathogenesis of periodontal disease and wound healing, the need for pocket elimination surgery was questioned. Surgery was performed to gain direct access to the root surface and reshape and recontour gingival tissues and/or bone to assist patients with access when performing daily home care procedures. Access flap techniques included the Widman Flap, Neumann Flap, apically positioned flap procedures and modified Widman Flap procedures (Heitz-Mayfield *et al.*, 2013).

The shift towards minimally invasive periodontal surgical procedures has somewhat changed periodontal surgery with use of illumination, magnification and microsurgical techniques resulting in better wound healing and less recession (Cortellini *et al.*, 2009, 2011; Graziani *et al.*, 2012; Retzepi *et al.*, 2007; Trombelli *et al.*, 2012). Papilla preservation flap surgery was found to be more beneficial as compared to access flap surgery for treatment of intrabony defects especially with clinical attachment gain (Graziani *et al.*, 2012).

Many studies have assessed the effect of non-surgical versus surgical therapy in pocket depth reduction and clinical attachment gains. Isidor *et al.*, (1984) found that reductions in pocket depths were achieved after RP although shallower pockets were recorded after the modified Widman flap (MW) and reverse bevel flap surgery. RP also resulted in slightly more attachment gain as compared to the surgical procedures (Isidor *et al.*, 1984). More sites with pocket depths greater than 6 mm were found after non-surgical therapy as compared to surgical therapy in the Lindhe and Nyman 1995 study. BOP were also observed in these deep sites that were treated non-surgically (Lindhe *et al.*, 1985).

Kaldahl *et al.*, (1996) included eighty-two patients in a split mouth design with coronal scaling (CS), RP, MW and flap with osseous resection surgery (FO). The results of this study found that all therapies resulted in pocket depth reduction. For PPDs between 1-4 mm, no differences were observed between the different treatment modalities at the

end of year two. At the end of year three, no differences between MW and RP were observed for pocket depths 5-6 mm and in pocket depths ≥ 7 mm by the end of year 5. FO produced the greater reduction in PPDs through year seven of supportive periodontal maintenance. Slight gains in clinical attachment levels were observed in sites with PPDs of 1-4 mm following CS and RP. In pocket depths 5-6 mm, RP and MW had more attachment gain as compared to CS and FO but the magnitude of the difference decreased with continuous SPT. In pocket depths ≥ 7 mm, clinical attachment gains were observed with RP, MW and FO. FO produced the most recession as compared to the other treatment modalities, followed by MW, RP and CS (Kaldahl *et al.*, 1996a).

The systematic review by Heitz-Mayfield *et al.*, in 2002 evaluated the effectiveness of surgical versus non-surgical periodontal therapy for the treatment of chronic periodontitis. This review included six randomized controlled studies from 1992 to 1996 (Isidor *et al.*, 1984; Kaldahl *et al.*, 1996a; Lindhe *et al.*, 1985; Lindhe *et al.*, 1982; Pihlstrom *et al.*, 1983; Ramfjord *et al.*, 1987). The results of this meta-analysis revealed that 12 months following treatment, in pocket depths >6 mm, 0.6 mm more probing depths reduction and 0.2 mm more clinical attachment gain was seen following surgical therapy as compared to non-surgical therapy. However, in pockets depths 4-6 mm, non-surgical therapy resulted in 0.4 mm more attachment gain and 0.4 mm less probing depth reduction as compared to surgical therapy. For pocket depths 1-3 mm, non-surgical therapy resulted in 0.5 mm less clinical attachment loss as compared with surgical therapy. Both treatment modalities are effective in treatment of chronic periodontitis; however, in deeper pockets >6 mm, surgical therapy was more effective in pocket depth reduction and gains in clinical attachment levels (Heitz-Mayfield *et al.*, 2002).

Periodontal surgery can be beneficial, however post-operative home care is important, and the outcomes of surgical interventions are dependent on patient's compliance and home care. Nyman *et al.*, (1977) conducted a study on 25 patients where patients were given OHI once before four types of surgical periodontal surgery. This study showed that oral hygiene habits were only temporarily improved, and patients had recurrence of plaque in surgical sites which reverted to an unstable periodontium. Surgical

procedures performed were found to be unsuccessful in preventing the recurrence of destructive periodontitis (Nyman *et al.*, 1977).

Smoking can affect wound healing following periodontal surgery and less favourable outcomes with regards to reduction in PPDs and clinical attachment gain may be observed in smokers as compared to non-smokers (Ah *et al.*, 1994) (Javed *et al.*, 2012; Preber *et al.*, 1990). Studies have found that more gingival recession can occur following surgical procedures as compared to non-surgical methods. However, some studies have found that these differences in recession decrease over time due to the coronal rebound of the soft tissue following periodontal surgery (Becker *et al.*, 2001; Kaldahl *et al.*, 1996a). The study by Matthews *et al.*, (1993) revealed that significantly more “post-operative discomfort” was experienced by patients following surgical periodontal therapy as compared to non-surgical therapy (Matthews *et al.*, 1993).

1.7.9 Periodontal Endoscopy: A Nonsurgical Approach

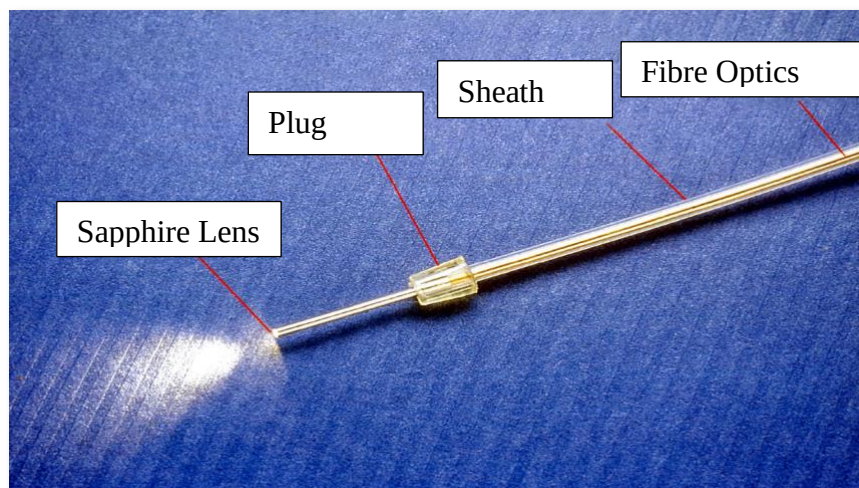
Traditional non-surgical periodontal therapy using closed subgingival SRD has been the main treatment option for patients with periodontal disease and has been found to improve periodontal health. However, the end point is usually difficult to determine with the use of tactile methods often not providing satisfactory information as to whether the biofilm has been completely removed. Various factors such as burnished calculus, root anomalies, anatomical factors affect the desired endpoint to be achieved. A clean root surface free of calculus and debris is the ideal endpoint, however the lack of visualization makes it difficult to realize if this has been achieved.

Periodontal endoscopy, as part of the nonsurgical approach for the treatment of periodontal disease, was introduced in 2001. The development of the periodontal endoscope has provided a method of directly visualizing root surfaces during periodontal debridement in an intact pocket without the need for surgical incision (Harrel *et al.*, 2015).

1.7.9.1 Periodontal Endoscopy Technology

Perioscopy® features miniaturized digital video technology allowing the operator to directly visualise the subgingival environment and at the same time remove any calculus or debris from the root surface. The endoscope consists of glass fibres (less than 1 mm in diameter) embedded in a plastic disposable sheath with a small stainless-steel tube and sealed sapphire lens. (Figure 1.6) There are several thousand optical glass fibers, of which some illuminate the subgingival environment, while others capture the image of the area being investigated (Harrel *et al.*, 2015).

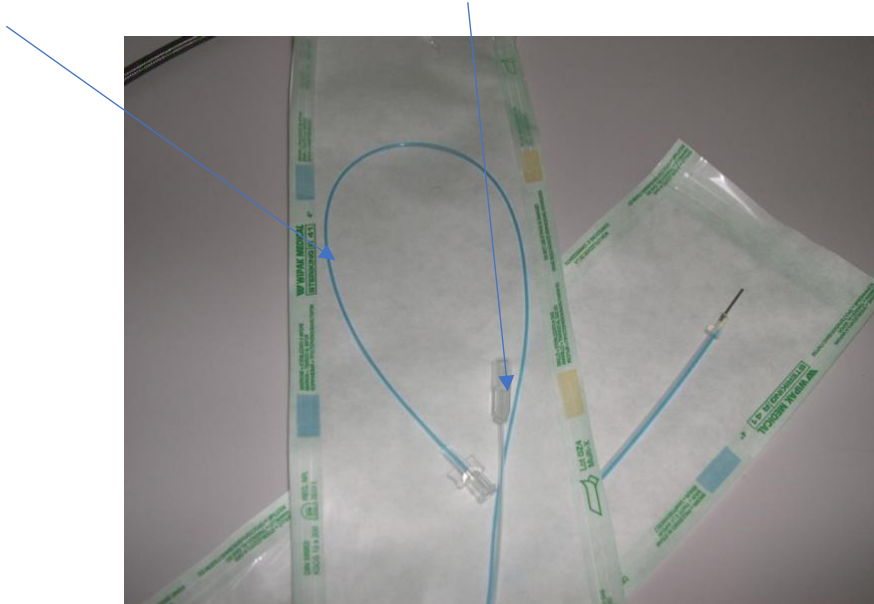
Figure 1. 6 Perioscope fibre



A disposable plastic sheath isolates the fibre from the environment. It has two tubes, one for the fibre and one for water which helps keep the subgingival area free of blood and debris. (Figure1.7)

Figure 1. 7 Perioscope sheath

Blue tube is the water line and the clear tube is the visual fibre line



The fibre is placed into an instrument called an explorer. The explorer is placed subgingivally and pushes the soft tissue away from the camera lens. (Figure1.8). There are straight and angulated explorers available for use. They can also be bent allowing access to surfaces around the teeth.

Figure 1. 8 Perioscope explorers



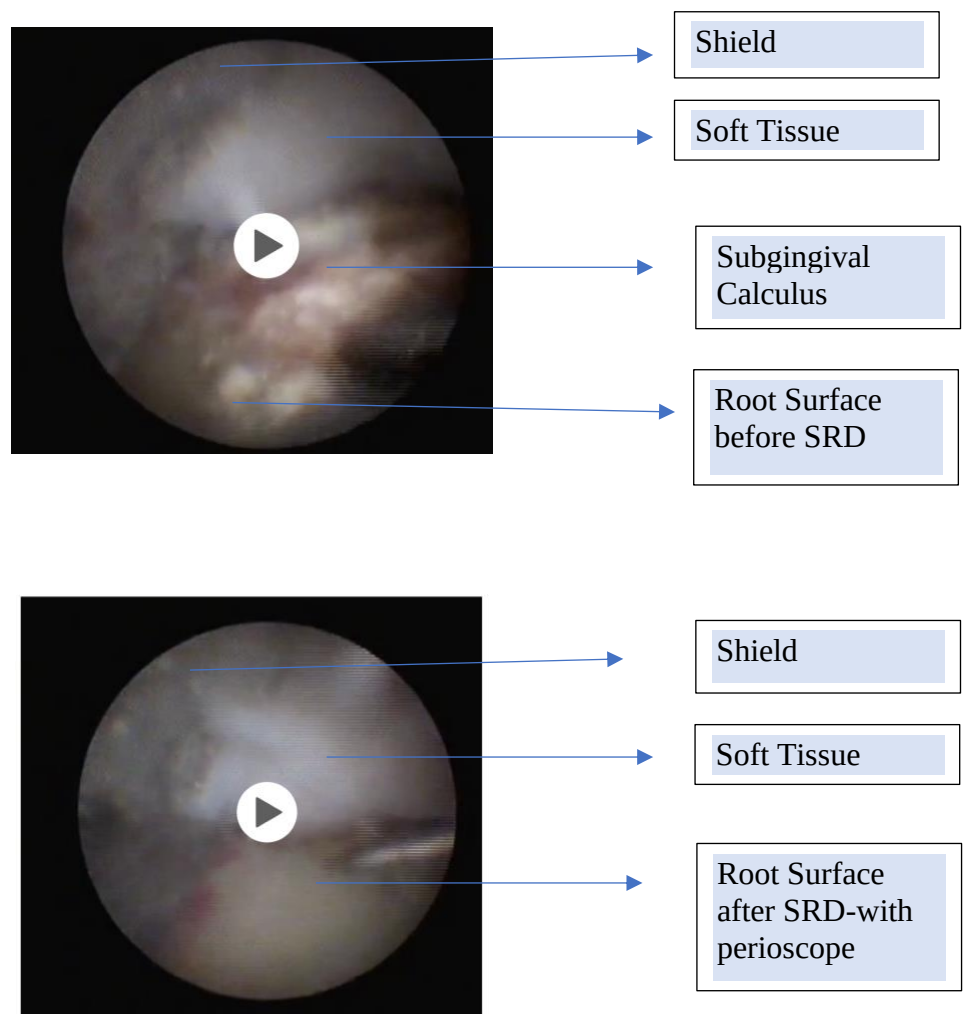
The image is shown on a monitor, magnified 24-48 times displaying minute details of the root surface. (Figure 1.9) The magnification is dependent on the distance of the object from the tip of the endoscope. The clinician is able to view the subgingival area, inflamed pocket wall as well as the subgingival calculus and biofilm deposits. Scaling instruments are inserted into the pocket to remove visible root deposits (Stambaugh *et al.*, 2002; Wilson *et al.*, 2008a). Figure 1.10 shows images before and after debridement using the perioscope.

Figure 1. 9 The Perioscopy system ®



(Harrel *et al.*, 2015)

Figure 1. 10 Images before and after SRD with perioscope



1.7.9.2 Clinical Trials Utilizing Periodontal Endoscopy

Since its introduction as a tool for nonsurgical periodontal therapy, there have been a number of studies conducted utilizing the Perioscope.

Improvements in clinical outcomes following SRD with the use of the periodontal endoscope were reported in many of the clinical trials (Kwan *et al.*, 2009; Stambaugh *et al.*, 2003; Stambaugh *et al.*, 2000). However, many of these studies had no control group for comparison. The retrospective study conducted by Stambaugh in 2003 examined perioscopy treatment outcomes after 3 years in 626 sites. The study showed that in pockets 4-6 mm, pocket reduction was 1.94 mm with endoscope aided SRD as compared with traditional SRD reported in literature of 1.0 mm. Attachment gain of 1.92 mm were reported utilizing endoscope SRD as compared to traditional SRD reported in literature of 0.38 mm. In pockets over 6 mm, a 4 mm pocket reduction was recorded in endoscope SRD as compared with conventional treatment reported in literature of 2.18 mm. Attachment gain was 4.1 mm in pocket depths over 6 mm as compared to 0.97 mm for traditional SRD (Stambaugh *et al.*, 2003).

A few of the studies included a control group for comparison between SRD only and SRD with the use of the perioscope. These were split-mouth design RCTs or parallel design RCTs. The pilot study by Blue *et al.*, (2013) found that both BOP and GI reduced at the perioscope sites; however no statistically significant differences were observed between the two groups with regards to PPDs and clinical attachment levels. PPDs reduced from 5.29 ± 0.4 mm at baseline to 3.55 ± 0.8 mm at visit 2 in the perioscope sites and 5.39 ± 0.5 mm to 3.83 ± 1.2 mm in the non-perioscope sites (Blue *et al.*, 2013). Hou (2016) reported reductions in PPDs and BOP in both SRD-only and SRD-with perioscope groups with no significant differences between the groups (Hou.Y, 2016). Liao *et al.*, (2016) found the use of the perioscope was effective in reducing deep pockets ≥ 6 mm in anterior teeth (Liao *et al.*, 2016). Avradopoulos *et al.*, (2004) did not find any statistically significant differences between the perioscope sites and the control sites with regards to PPD reduction. At one month, 50% of the control sites had a decrease of ≥ 2 mm in PPDs, compared to 55% in the test group. When comparing baseline and three months, 55% of control sites had a decrease in PPDs of >2 mm as

compared to 46% of the test sites. No statistical significant differences were observed for inflammatory markers (PGE₂) levels between test and control sites at one and three months (Avradopoulos *et al.*, 2004).

The periodontal endoscope has been shown to be a worthy diagnostic tool for detection of residual calculus and also caries detection. Stambaugh *et al.*, (2002) found that 95% of all root surface deposits and caries were detected with the perioscope (Stambaugh *et al.*, 2002). Their study in 2000 on 42 teeth and 210 sites found that endoscope SRD was more effective in removing subgingival deposits as compared to conventional treatment. Only 1.2% of endoscope aided SRD had residual calculus as compared to similar study designs in literature that report that 10-50% of residual calculus remains following traditional SRD (Stambaugh *et al.*, 2000). A stereomicroscope and digital image analysis were used to determine the amount of residual calculus on extracted teeth after SRD with or without the endoscope. This study found that teeth that were scaled using conventional methods had 2.14% more residual calculus. In deeper pockets, less residual calculus was found in teeth treated using the endoscope (Geisinger *et al.*, 2007). Michaud *et al.*, (2007) also reported that there was 1.16% less residual calculus at perioscope treated sites as compared to control sites with significantly less calculus (2.63%) at interproximal sites as compared to the control group ($p=0.003$). Shallow sites with PPDs ≤ 6 mm also had less residual calculus in the test group as compared to the control group. However, calculus removal in multi-rooted teeth was not found to be better in perioscope treated sites (Michaud *et al.*, 2007). Similarly, studies by Hu-Rui *et al.*, (2013) and Zhou (2016) reported less residual calculus following debridement with the perioscope as compared to conventional SRD, although no statistical differences were observed for these studies (Hu-Rui *et al.*, 2013; Zhou, 2016).

When the perioscope was compared to a conventional tactile explorer to determine levels of subgingival calculus, the perioscope was found to be superior and was able to detect significantly more calculus than the explorer (Osborn *et al.*, 2014).

A pilot study conducted in 2008 evaluated the histologic response of the level of inflammation following treatment of patients with severe chronic periodontitis with an endoscope. Twelve teeth from six patients were treated with the dental endoscope and all visible subgingival deposits were removed. Six months after treatment with the

endoscope, the teeth as well as the coronal portion of the periodontal attachment apparatus was removed and sent for histological examination. No histologic signs of chronic inflammation were seen on previously diseased root surfaces, bone repair and growth of the long junctional epithelium was observed (Wilson *et al.*, 2008b).

Wilson *et al.*, (2008) conducted a study on 26 patients with moderate to severe periodontitis. This study endoscopically explored the relationship between subgingival calculus and biofilm and presence of visible inflammation (redness) on the pocket wall and related these to traditional periodontal indices. At the initial visit, indices such as GI, probing depths, clinical attachment levels were taken at six sites per tooth. To compare these with endoscopic findings, indices such as endoscopic biofilm index (EBI), endoscopic inflammation index (EII), and the endoscopic calculus index (ECI) were used. A stent was used ensuring that the endoscopic explorer followed the same pathway as the periodontal probe. This study demonstrated that biofilm and calculus often co-existed in the same path and increased inflammation was observed in areas of calculus deposits covered with biofilm. A statistically significant relationship was found between subgingival calculus index (SCI), subgingival biofilm index (SBI) and subgingival inflammation on the pocket wall. With the use of the endoscope, it was found that subgingival inflammation was more prevalent when biofilm and calculus was detected. Biofilm and calculus were observed in >60% of the sites where inflammation was detected, indicating that calculus may be linked with subgingival inflammation. In this study, there was no significant relationship between subgingival inflammation, GI and presence of BOP; however, it was found that deeper pockets were related to subgingival inflammatory changes (Wilson *et al.*, 2008a).

A direct relationship between BOP and presence and amount of subgingival deposits were demonstrated in the Checchi *et al.*, (2009) study. A linear association between BOP and PPD, EBI, and ECI was observed. A level of specificity was observed with BOP but low sensitivity with ECI (sensitivity 40%, specificity 86%) and EBI (sensitivity 37%, specificity 89%) (Checchi *et al.*, 2009).

Teeth with furcation involvement, maxillary bicusps and the distal areas of second molars did not respond favourably with use of the periodontal endoscope (Stambaugh

et al., 2003). Anatomy and difficulty with access when using the endoscope could be reasons for this.

Previous research utilizing the periodontal endoscope have examined the use of this tool in improving clinical parameters such as PPD, PAL, BOP and GI. The studies by Stambaugh (2000,2003) and Kwan and Workman (2009) had no control group for comparison. Other studies were RCT patient matched, split mouth design or parallel design (Avradopoulos *et al.*, 2004; Blue *et al.*, 2013; Hou.Y, 2016; Liao *et al.*, 2016). Kwan and Workman (2009) in this case series did include radiographic analyses; however, there was no indication whether these radiographs were standardized (Harrel *et al.*, 2015; Kwan *et al.*, 2009). There has been no research utilizing standardised radiographs to examine changes in RBLs post-perioscopy. Microbiological changes following non-surgical periodontal therapy have been researched over the years, but there have been no studies to date examining the changes in numbers and levels of periodontal pathogens following non-surgical debridement with the use of the periodontal endoscope. The current study seeks to obtain data which will help to address these research gaps.

The tables below provide a summary of these clinical trials.:

Table 1.1 includes studies examining the effect of use of periodontal endoscopy on clinical parameters.

Table 1.2 includes a summary of clinical trials utilising periodontal endoscopy as a diagnostic tool to examine percentage of residual calculus and histologic response following endoscopic debridement.

Table 1. 1 Clinical Trials examining the effect of use of periodontal endoscopy on clinical parameters							
Visualization of Submarginal Gingival Root Surfaces with the Dental Endoscope(Stambaugh <i>et al.</i> , 2000)							
Study Design	Aim	Treatment Protocol	F/Up*	No* of subjects	Outcome measures	Results	Limitations
Retrospective preliminary trial	To determine changes in clinical parameters following use of the endoscope	Included patients in periodontal maintenance who were not been maintained and had AL, bleeding and inflammation. Two groups A and B. Group A included all 8 patients. Group B included sites that demonstrated 2 mm or more of AL within 2 years	2 years	8 subjects	PPDs, Probing attachment gain, gingival inflammation and bleeding scores	All patients had subgingival deposits. After debridement with the endoscope both groups had improvements in gingival inflammation, bleeding scores, probing depths and attachment gain.	No control group for comparison. Small sample used.

Improved CAL and PPD with Endoscope-aided Scaling and Root Planing(Stambaugh <i>et al.</i> , 2003).							
Study Design	Aim	Treatment Protocol	F/Up	No* of subjects	Outcome measures	Results	Limitations of study
Retrospective	Examined perioscope treatment outcomes 3 years post-therapy	Patients were treated with periodontal endoscopy	3 years	N/A	PPDs, Probing attachment gain	PPD reduction was 1.94 mm for PPDs 4-6 mm with endoscope aided SRP as compared to 1.0 mm reported in the literature for traditional SRP. Attachment gain of 1.92 mm as compared to 0.38 mm for traditional SRP reported in literature. For PPDs over 6 mm, a 4 mm PPD reduction as compared with conventional	No control group for comparison

						treatment reported in literature of 2.18 mm. Attachment gain was 4.1 mm in pocket depths over 6 mm as compared to 0.97 mm for traditional SRD.	
Clinical and inflammatory evaluation of Perioscopy on patients with chronic periodontitis(Avradopoulos <i>et al.</i>, 2004)							
Study Design	Aim	Treatment Protocol	F/Up	No* subjects/ of teeth/sites	Outcome measures	Results	Limitations of study
RCT within-patient, matched- site design, Pilot Study	To evaluate the clinical and inflammatory changes produced in chronic, non-responsive periodontitis sites when comparing SRD alone and SRD with perioscope.	Inclusion criteria 6-8 periodontal pockets measuring 5-8 mm. Half of the sites were treated with SRD and other half with SRD - with the use of the perioscope. Patients completed initial phase therapy and continued with SPT for 1 year.	1 month and 3 months	7 patients (1 lost to follow) 44 teeth sides Age: 45-51 years	PPDs and Inflammatory markers	At one month, 50% of the control sites had decrease of ≥ 2 mm in PPDs, compared to 55 % in the test group. When comparing baseline and three months, 55% of control sites had a decrease in PPDs of > 2 mm as compared to 46% of the test sites. No statistically significant decreases were found between the test and control sites. No statistically significant differences were observed for inflammatory markers (PGE ₂) levels between test and control sites at one and three months.	Treatment time was too long for debridement using the perioscope (45minutes to more than 1 hour for an experimental site). Experience of operator and training needs to be considered. Longer re-evaluation time is recommended, 1 month and 3 months is too short.
Micro Ultrasonic Endoscopic Periodontal Debridement: Retrospective Analysis of Treatment with at least 1 year Follow-up (Kwan <i>et al.</i>, 2009)							
Study Design	Aim	Treatment Protocol	F/Up	No* subjects/ of	Outcome measures	Results	Limitations

				teeth/sites			of study
Non-Blinded Prospective Study	To determine changes in PPDs following the use of endoscopic debridement	All patients were treated using the perioscope in one visit. Patients were given antibiotics either before or immediately following treatment. Antibiotics included either metronidazole 500mg bid × 7 days, or metronidazole and amoxicillin both 500 mg bid × 7 days, or azithromycin 500mg qd×3days.	1 year Patients were seen every 3 months for SPT. PPD was recorded and compared to baseline 1 year following treatment	270 subjects <u>Molar Teeth</u> 45 with PPDs 10-11 mm; 284 with PPDs 7-9 mm 478 with PPDs 5-6 mm <u>Premolar Teeth</u> 8 with PPDs 10-11 mm 30 with PPDs 7-9 mm; 266 with PPDs 5-6 mm <u>Anterior Teeth</u> 7 with PPDs 10-11 mm 57 with PPDs 7-9 mm 246 with PPDs 5-6mm	PPDs	<u>Molar Teeth</u> PPDs 10-11 mm (71% reduced to 6–9 mm; 20% reduced to ≤5 mm) PPDs 7-9 mm (55% reduced to ≤5 mm) PPDs 5-6 mm (69% reduced to ≤4 mm) <u>Premolar Teeth</u> PPDs 10-11 mm (38% reduced to ≤5 mm) PPDs 7-9 mm (57% reduced to ≤5 mm) PPDs 5-6 mm (78% reduced to ≤4 mm) <u>Anterior Teeth</u> PPDs 10-11 mm (71% reduced to ≤5 mm) PPDs 7-9 mm (89% reduced to ≤5 mm) PPDs 5-6 mm (92% reduced to ≤4 mm)	No control group for comparison, non-blinded

The relationship between bleeding on probing and subgingival deposits. An endoscopic evaluation(Checchi <i>et al.</i> , 2009)							
Study Design	Aim	Treatment Protocol	F/Up	No subjects/ of teeth/sites	Outcome measures	Results	Limitations of study
Observational	The aim of this study was the examine the presence of subgingival deposits and evaluate their correlation with BOP and other periodontal clinical parameters using the periodontal endoscope.	Patients received conventional SRD under local anaesthesia. OHI and motivation were included. One month later, a re-evaluation appointment was conducted where endoscopic data was recorded by random selection. Two examiners collected the data. They were blinded by each other's findings. Six sites were evaluated on each tooth using a periodontal probe (examiner A) and under endoscopic evaluation (Examiner B). Third molars were excluded. Time interval between two examinations were 15 minutes.	1 month	16 patients (9 females, 7 males) 107 teeth, 642 sites Age:45 – 54 years	PI,GI, PPDs, and BOP. EBI, and ECI	A direct relationship between BOP and presence and amount of subgingival deposits were demonstrated. A linear association between BOP and PD, EBI, and ECI was observed. A level of specificity was observed with BOP but low sensitivity with ECI (sensitivity 40%, specificity 86%) and EBI (sensitivity 37%, specificity 89%).	A low correlation between GI and BOP was observed, probably due the study design. No correlation was found between PI and BOP, which could be explained by patient's personal oral hygiene. Also, the PI did not indicate the amount of plaque but rather the presence or absence of plaque.
A pilot study comparing the outcome of scaling/root planing with and without Perioscope technology(Blue <i>et al.</i> , 2013)							
Study Design	Aim	Treatment Protocol	F/Up	No subjects/ of teeth/sites	Outcome measures	Results	Limitations of study

RCT split mouth design, Pilot Study	The purpose of this study was to determine if the use of a periodontal endoscope improves periodontal outcomes of SRP when compared to SRP alone	Sites within 2 quadrants of each subject were treated with or without the use of the Perioscope.	6-8 weeks and 3 months	26 with moderate periodontitis 364 sides Age: ≥20 years	PPDs, GI and BOP	BOP and GI reduced in the perioscope sites at visit 1 and visit 2. PPDs and gain in clinical attachment levels were observed for all sites, with no significant differences found between the control and test sites. From baseline to visit 2, reductions in mean probing depth (SD) from 5.29 mm (0.4) to 3.55 mm (0.8) was observed in the Perioscope sites and 5.39 mm (0.5) to 3.83 mm (1.2) in non-Perioscope sites. The adjunctive use of the perioscope resulted in statistically significant improvements in both in gingival inflammation and BOP.	Differing results with patients with different levels of disease. Operator experience
Comparison of the effects of periodontal scaling under the periodontal endoscope and traditional periodontal scaling on periodontitis(Hou.Y, 2016)							
Study Design	Aim	Treatment Protocol	F/Up	No subjects	of Outcome measures	Results	Limitations
RCT, parallel design	Compared the clinical outcomes of patients with periodontitis who had been treated by means of periodontal endoscopy or traditional SRD	Sites were treated with periodontal endoscopy or traditional SRD	Three months	48 Age: 38-63 years	PPDs, BOP	Reduction in PPDs and BOP were observed in the group treated by periodontal endoscopy however no significant differences were observed between the two groups.	Short f/up
A clinical evaluation of periodontal treatment effect using periodontal endoscope for patients with periodontitis: a split-mouth controlled study(Liao et al., 2016)							

Study Design	Aim	Treatment Protocol	F/Up	No subjects/ of sites	Outcome measures	Results	Limitations
RCT split mouth design	Compared the clinical outcomes of patients with periodontitis who had been treated by means of periodontal endoscopy or traditional SRD.	Patients with moderate to advanced periodontal destruction were treated with SRP in a split-mouth design randomly with the periodontal endoscope (test group) or without (control group)	6 weeks and 3 months	11 subjects 696 sites	PI, BI, Probing depth (PD) and AL	Significant reductions in PD, BI and AL values at 6 weeks and 3 months after treatment ($P < 0.001$) were observed in both groups. For sites with $PPD \geq 6$ mm at baseline, in the test group, the PD value (4.0 ± 1.2 mm) and AL value (3.8 ± 0.9 mm) at the end of 3 months were significantly lower than that at the end of 6 weeks (PD 4.4 ± 1.3 mm, $P < 0.001$; AL (4.1 ± 1.1) mm, $P < 0.05$). No statistically differences between two groups at baseline, at the end of 6 weeks and 3 months were seen. For anterior teeth, with $PPDs \geq 6$ mm, at the 3month appointment, the test group had a significantly lower PPD as compared to the group. (3.2 ± 0.9) mm vs (3.7 ± 0.9) mm, $P < 0.05$. The AL was also lower in the test group (2.9 ± 1.2) mm vs (3.6 ± 1.3) mm, $P = 0.061$).	Short f/up Small sample used

						The use of the perioscope was found to be effective more especially for the medium and long-term prognosis of deep pockets and single rooted teeth.	

- F/Up – Follow Up
- No- Number

Table 1. 2 Clinical Trials utilising periodontal endoscopy to examine percentage of residual calculus detection and histologic responses					
Endoscopic visualization of the submarginal gingiva dental sulcus and tooth root surfaces(Stambaugh <i>et al.</i> , 2002)					
Aim	Treatment Protocol	No* of sites	Outcome Measures	Results	Limitations
To develop the skills of clinicians in using fibre optics to visualise the subgingival sulcus. To evaluate teeth with respect to root deposits and caries using the endoscope	Teeth were evaluated and scored for root deposits and caries. The teeth were extracted, and the 2 scores compared.	42 teeth, 210 sites	Detection of root deposits and caries	Ninety five percent of all root surface deposits, and caries were detected with the use of the endoscope.	N/A
The effectiveness of subgingival scaling and root planing: an evaluation of therapy with and without the use of the periodontal endoscope(Geisinger <i>et al.</i> , 2007)					
Study Design/Aim	Treatment Protocol	No* of sites	Outcome Measures	Results	Limitations
The purpose of this RCT study was to determine whether the adjunctive use of the periodontal endoscope with SRP resulted in a reduction in residual subgingival calculus after treatment compared to SRP alone.	Each tooth pair was randomized to receive either SRP. alone or SRP with the endoscope. Teeth were then extracted and with use of stereomicroscope and digital image analysis, the percentage of residual calculus was determined.	15 subjects (aged 40-73 years) 50 tooth pairs (single rooted teeth)	Percentage of residual calculus post treatment, Average treatment time	Significantly more residual calculus was found in the SRP only group ($p<0.001$). Mean differences in residual calculus was 1.30%($p,0.015$) and 2.93%($p<0.001$) at the buccal/lingual and interproximal areas respectively. Deeper sites had significantly less residual calculus in the SRP with perioscope group as compared to SRP only. No differences were detected in shallower pockets between groups. Treatment time decreased as operator experience increased.	N/A

The efficacy of subgingival calculus removal with endoscopy-aided scaling and root planing: a study on multirooted teeth(Michaud <i>et al.</i> , 2007)					
Study Design/Aim	Treatment Protocol	No* of subjects/sites	Outcome Measures	Results	Limitations
To determine if SRD with the use of the endoscope resulted in a greater reduction of residual calculus compared to SRD alone in multirooted teeth. RCT, tooth pair	The test group teeth were scaled with the use of the endoscope. The end point was no visible calculus visualised on the LCD screen. The control group teeth were scaled, and root planed using conventional methods. The end point was determined by a smooth root surface and residual calculus been detected with a number 3 cow horn explorer. Maximum time for treatment for both groups was 60 minutes.	24subjects Age: 38-73 years 70 Multi-rooted teeth	Percentage of residual calculus and average treatment time	There was 1.16% (P = 0.097) less residual calculus at test versus control sites. In the test group, root surfaces had 2.63% less residual calculus at interproximal sites as compared to the control group (p=0.003). More residual calculus was detected in the test group in the buccal/lingual surfaces as compared to the control group (0.36%; p = 0.652). No statistically significant differences were found between groups in residual calculus at deeper probing depths or at sites with deep furcation. At shallower interproximal sites with probing depths ≤6 mm, significantly less residual calculus was detected in root in the test group as compared to the control group (p = 0.020). As operator experience improved, the treatment time decreased. However, more experience did not result in improvement in residual calculus levels. This study found no significant improvement in calculus removal in multi-rooted teeth with the use of the endoscope as compared to conventional debridement.	Teeth included were considered periodontally hopeless producing a difficult environment to use the endoscope. Bleeding interfered with the image visible. The presence of granulation tissue made it difficult to retract with the probe. The appearance of subgingival calculus made it difficult visualise all calculus present.

Absence of histologic signs of chronic inflammation following closed subgingival scaling and root planing using the dental endoscope: human biopsies - a pilot study(Wilson <i>et al.</i> , 2008b)					
Study Design/Aim	Treatment Protocol	No of subjects/sites	Outcome Measures	Results	Limitations
Observation Study. To evaluate the histologic response (i.e., level of inflammation) following treatment of severe chronic periodontitis with a dental endoscope.	Inclusion criteria: Patients with severe, chronic periodontitis with study sites with PPDs ≥ 7 mm. Teeth were treatment planned for extraction. Patients took amoxicillin, 1 g, 1 hour prior to treatment continue with the antibiotic for 8 days. Patients were instructed to rinse with 0.12% chlorhexidine digluconate for 30 seconds prior to the procedure. They continued with the mouth rinse twice daily for 6 weeks. The test teeth were anaesthetised and were endoscopically evaluated for the presence of subgingival calculus. A groove was made with a #1 round bur in a low-speed handpiece. The relationship of the groove and calculus deposits was recorded using a digital video recorder and this assisted with the eventual histologic evaluation of the teeth. The 12 teeth and coronal portion of the periodontal attachment were removed and 6 months later and	6 patients Age: 46-63 years 12 teeth	Histologic Changes and level of inflammation	The results of this study showed that there were no histologic signs of chronic inflammation following a single treatment of subgingival SRP with the use of the dental endoscope. Only one section of the one tooth had deposits of calculus and biofilm, however it was thought to be deposited after initial phase therapy. Bone repair and the growth of a long junctional epithelium were also observed.	Small sample size

	sent for histological examination. A radiograph was taken. The hard tissue was sent for histologic testing. Patients were seen weekly for the 1st month following treatment for OHI. After the 1st month, patient were recalled monthly for supragingival polishing.				
The relationship between the presence of tooth-borne subgingival deposits and inflammation found with a dental endoscope(Wilson <i>et al.</i>, 2008a)					
Study Design/Aim	Treatment Protocol	No of subjects/sites	Outcome Measures	Results	Limitations
Observation Study To explore the relationship between subgingival calculus and biofilm and the presence of endoscopically visible signs of inflammation on the pocket wall and the relationship of these deposits to periodontal indices.	Included standardised masked examiners. GI, probing depths, gingival recession and clinical attachment levels were recorded by 1st examiner. The 2nd examiner used a dental endoscope and recorded indices including an EBI, ECI, and endoscopic GI. To ensure consistency that the periodontal probe and endoscope explorer travelled along the same path, a fixation stent was used.	26 patients with moderate to severe periodontitis Twelve teeth	GI, probing depth, gingival recession, and clinical attachment level. EBI, ECI, and endoscopic gingival index	A statistically significant relationship were reported between subgingival calculus covered with biofilm and inflammation of the pocket wall measured by redness of the pocket epithelium. This inflammation was associated with biofilm over deposits of calculus, not biofilm alone in more than 60% of the cases. Subgingival calculus was statistically significant in relation to the positive traditional GI.	N/A
Effect of plaque and calculus removal by scaling and root planing with or without perioscopy in chronic periodontitis(Hu-Rui <i>et al.</i>, 2013)					
Study Design/Aim	Treatment Protocol	No of subjects/sites	Outcome Measures	Results	Limitations
RCT parallel design Comparing SRD only with SRD with perioscope with	Maximum time for treatment was 10 minutes per tooth. Teeth were extracted and examined using computer software to assess	16 subjects Age 42-71 years	Percentage of Residual Calculus	Less residual calculus was found in teeth treated with perioscope as compared to conventional treatment, however no significant differences were observed between the two groups.	N/A

regards to percentage of residual calculus following treatment	percentage of residual calculus with both treatment methods used	26 single rooted teeth			
Endoscopic vs. tactile evaluation of subgingival calculus(Osborn <i>et al.</i>, 2014)					
Study Design/Aim	Treatment Protocol	No of subjects/sites	Outcome Measures	Results	Limitations
A randomized split mouth design Comparing the periodontal and calculus changes with and without the use of a Perioscope	Inclusion criteria: Each participant was required to have a minimum of 4 individual periodontal sites of pocket depth measurement >5 mm in 2 separate quadrants. All 26 participants underwent SRP. After baseline subjects underwent full mouth scaling and root planning. The perioscope was used for calculus removal in the treatment quadrant but not in the control quadrant. One quadrant was examined for tactile calculus detection and the other quadrant examine with tactile detection plus the perioscope. Calculus index was scored from 0-3 and recorded at baseline, and 2 months post SRD. Sites with calculus at visit 1 were retreated. The ODU 11-12 explorer was used for calculus detection. Two subsequent 6-8 week re-evaluation appointments were done. Periodontal and calculus parameters were measured at visit 1 and visit 2.	Adult subjects 26 participants 202 perioscope study sites and 162 non-perioscope sites	Pocket depth, attachment level, bleeding, gingival health and calculus index were taken at study sites in both quadrants before treatment and at re-evaluation. In the treatment group, the level of calculus was assessed using the ECI.	The use of the perioscope resulted in significantly more calculus being detected versus the tactile explorer for all 3 subject visits ($p<0.005$). A statistically significant difference was found for the mean changes (reduction) in calculus detection from baseline to visit 1 for both the Perioscope quadrants and tactile quadrants ($p<0.0001$). However, from visit 1 to visit 2, the perioscope quadrants had further reductions in calculus detections($p<0.025$), suggesting that the use of the endoscope was more efficient in calculus detection. This study supported the use of the periodontal endoscope in the calculus detection more especially in the re-evaluation phase of periodontal therapy.	Varying levels of periodontal disease could affect outcomes. The method of obtaining the calculus indices included visual (perioscope)and tactile(explorer), which could result in errors in consistency and comparison. Although training and calibration occurred prior to the study with use of the perioscope, operator experience can affect the results.

The application of periodontal endoscope to remove subgingival calculus(Zhou, 2016)					
Study Design/Aim	Treatment Protocol	No of subjects/sites	Outcome Measures	Results	Limitations
RCT parallel design To compare SRD only and SRD with perioscope with regards to percentage of residual calculus	Teeth were debrided and then extracted and analysed for residual calculus using computer software	30 subjects Age: 33-51 years 50 Single Rooted Teeth	Percentage of Residual Calculus	The percentage of residual calculus was less in the SRD with perioscope sites as compared to the control sites, however this difference was not significant.	Only included single rooted teeth

- No - Number
- N/A – Not available

1.8 Supportive Periodontal Maintenance Therapy

The importance of continued SPT after active therapy has been emphasized over the years with several studies linking SPT together with good oral hygiene practices with arresting the progression of disease, reducing the rate of tooth loss and resulting in periodontal stability (Axelsson *et al.*, 1981; Badersten *et al.*, 1987a; Costa *et al.*, 2015; Costa *et al.*, 2012a; Costa *et al.*, 2012b; Kaldahl *et al.*, 1996b; Knowles *et al.*, 1979; Lindhe *et al.*, 1984; Pihlstrom *et al.*, 1983; Ramfjord *et al.*, 1975; Westfelt *et al.*, 1983). Long term SPT has been shown to influence periodontal prognosis and tooth survival (Cohen *et al.*, 2003; Farooqi *et al.*, 2015; Lee *et al.*, 2015). A mean annual loss of periodontal support of 0.03 mm were reported in well-maintained patients as compared to 0.1 mm in patients who received only one examination and no reinforcement of oral hygiene (Suomi *et al.*, 1971). The study by Axelsson *et al.*, (1981) on 90 patients with advanced periodontitis showed that patients who followed a strict maintenance program after initial treatment were able to maintain good oral hygiene standards, shallower pockets and unaltered attachment levels. In addition, no tooth loss was recorded after six years. Those patients who were not in a supervised program, showed signs of recurrent periodontitis, and some tooth loss after three and six years (Axelsson *et al.*, 1981). A review by Chambrone *et al.*, (2010) included studies where patients with periodontitis completed periodontal therapy and were placed on a maintenance program for at least five years. Of the 41,404 teeth present, 3919 teeth were lost after periodontal treatment. Factors such as age, smoking, tooth type, location and initial tooth prognosis were associated with tooth loss during periodontal maintenance. However, heterogeneity existed in studies analysed and definitive conclusions were difficult to establish (Chambrone *et al.*, 2010).

A retrospective study by Nibali *et al.*, (2016) included 100 patients who completed periodontal therapy and were followed for at least 5 years in SPT. This study found that the overall average tooth loss was only 0.06 teeth per patient/year and only 0.02 was for periodontal reasons. Factors associated with tooth loss included age, patient prognosis based on periodontal risk assessment system, tooth prognosis, furcation involvement, previous endodontic therapy, medical history and smoking histories. This study supported that patients in supportive periodontal maintenance are at a low risk of tooth loss (Nibali *et al.*, 2016).

The frequency for SPT can range between 3-4 months and this was based on previous studies that have reported periodontal stability following this frequency of recall (Lindhe *et al.*, 1975; Ramfjord *et al.*, 1973; Waerhaug, 1977). However, the frequency of SPT should be tailored to the individual needs of the patient. Patient compliance and identifying risk factors for tooth loss during periodontal maintenance therapy are imperative and can determine the frequency of visits.

Clinical examinations performed at the SPT visits may not necessarily include all the information obtained at the initial examination. At SPT visits, identifying areas of risk and disease progression are essential. Reinforcing home care procedures and discussing ways to improve oral hygiene practices should be included in this visit. Decisions should be made regarding changing or keeping the recall interval based on findings at the SPT appointment (Mombelli, 2019).

It is also important to give consideration to the new classification of periodontal disease which includes “Periodontal Health”. Patients with a reduced periodontium can be grouped into either “periodontal disease stability” or “periodontal disease remission/control”. “Periodontal disease stability” is when the periodontal disease has been treated and patients present with minimal BOP, improvements in PPD’s and PAL and there is no sign of progressive destruction. “Periodontal disease remission/control” can be described as reduction in inflammation and clinical parameters such as PPD and PAL but there is not total resolution (Lang *et al.*, 2018).

1.9 Private Practice Versus Hospital Based Research

Research in dentistry can be conducted in both academic/university-based settings and in private dental clinics. Some of the issues with research conducted in tertiary and academic hospitals is that it may not truly reflect the broad community diversity and dissimilarities in race, rural/urban status, health care coverage, health care access and health literacy may exist (Green *et al.*, 2001; White *et al.*, 1961). Private practice research can generate evidence based knowledge with good external validity as the research is usually conducted on patients seen in clinical practice (Gordan *et al.*, 2019).

Majority of dentistry takes place in private dental clinics in Australia with the total expenditure on dental services (except in hospital) reported as \$8,706 million in 2012-2013. The out of pocket expenses for individuals was 58% of this total cost (Chrisopoulos *et al.*, 2016). Research conducted in private dental settings can result in mutually beneficially end results. There are so many new advancements in dental research, and implementation of clinical trials in a private practice setting provides an opportunity to research new technologies and developments in the field of dentistry before it is offered to the broader community. Clinical trials in private practice also allows the patients the opportunity to pursue alternative treatment options.

Initiating a research project at private clinic can be less time consuming as compared to university-based projects. Funding and accessibility to materials required for research at a university or academic based institution can slow the progress of a research project. In addition, participants may not be compliant, and many can exit the research study. The compliance in a private dental facility may be much higher as participants are paying for the treatment provided and are more likely to attend.

The infrastructure of private practice including the equipment, computer programmes and staff, can facilitate the easy transition into turning a private practice into a research centre. The current research project was carried out at a specialist private practice. Patients were recruited from suburbs around Victoria and those that consented to be part of the research project were included. Patients paid for the treatment provided during the study period which could have contributed to the low numbers of “loss to follow” participants.

1.10 Study Aims

Studies conducted have shown the benefits of traditional non-surgical periodontal therapy and endoscope SRD in reducing clinical and inflammatory parameters; however, there is a need for more RCTs to compare these two treatment options and investigate if one is more advantageous than the other. Osseous changes can occur after nonsurgical periodontal therapy, but it is often difficult to determine if changes have occurred due to limitations with conventional radiography. This study included

standardized radiographs using customized positioning stents and paralleling x-ray holding devices. In addition, digital radiography and digital software will be used to determine areas of osseous change. The link between periodontal pathogens and progression of periodontal disease has been established and studies have shown that periodontal treatment can reduce bacterial levels. However, there is a need to investigate if endoscope SRD is more effective in reducing levels of periodontal pathogens as compared to conventional non-surgical treatment. In addition, we aim to investigate if bacterial counts are lowered, can they be maintained or reduced even further with strict three monthly supportive periodontal maintenance?

The main aims of this study are:

1. To compare endoscope- assisted SRD to traditional SRD in reducing clinical and inflammatory parameters in a 12-month period.
2. To record radiographic changes in test and control groups and to assess if endoscope assisted SRD shows more evidence of radiographic changes after the 12-month period as compared to conventional treatment.
3. To compare bacterial counts of 11 bacteria including, *A. actinomycetemcomitans*, the red complex bacteria consisting of *P. gingivalis*, *T. forsythia*, and *T. denticola*; the orange complex (*P. intermedia*; *P. micros*; *F. nucleatum/periodonticum* and the orange associated complex including *C. rectus* and *E. nodatum* as well as the green complex which consists of *E. corrodens* and *Capnocytophaga sp. (sputigena; gingivalis; ochracea)* in both groups at pre-treatment, 3 months and then 12-month appointments. The aim is to compare the reduction of microflora between test and control groups.
4. To determine the need for surgical intervention in both test and control groups after the 12-month treatment.

CHAPTER 2

METHODOLOGY AND STATISTICAL ANALYSIS OF DATA

This chapter describes the clinical and microbiological methods used for this study. The statistical methods used to analyse the data is also included.

2.1 Ethics Approval

Prior to the commencement of the study, human ethics approval was obtained from the University of Melbourne HEAG (Health Sciences Human Ethics Sub-Committee number 1543782). Subjects were informed of the study protocol and written informed consent was obtained from all participants.

2.2 Clinical Methodology

This section describes the methods employed for subject selection.

2.2.1 Sample Size

This study compared the effectiveness of SRD-only with SRD-with perioscope on clinical, microbiological and radiographic parameters. The sample size had to be set by considering both the practical constraints on obtaining participants and collecting data, and statistical considerations. A study by Blue *et al.*, had suggested that powering the study to detect a standardized effect size of one for the difference in pocket depth size; in that study the desired mean difference was 1.25 mm with a standard deviation (presumed to be within group) of the same magnitude, giving a standardised effect size of one (Blue *et al.*,2013). In the present study, with an independent samples design, to detect a standardized effect size of one, with a statistical significance level of 0.05 and power of 0.80, the target sample size in each group was 17. In practice, this was surpassed with 19 in each group.

2.2.2 Subject Selection

All subjects were recruited from the Perio Centre (Private Specialist Periodontal Practice, 401 Wattletree Road, East Malvern, Victoria). They were screened for suitability and, if accepted, they provided signed informed consent.

2.2.2.1 Inclusion Criteria

Subjects that were diagnosed with moderate to severe chronic periodontal disease, had at least 20 natural teeth, with more than ten sites with pocket depths greater than 4 mm were eligible for inclusion. This project commenced before the “2017 periodontal classification” was released, hence the 1999 periodontal classification was used.

2.2.2.2 Exclusion Criteria

Patients with medical conditions that required antibiotic coverage prior to any dental treatment; patients who have taken antibiotics in the last three months for medical reasons; patients whose medical conditions may contraindicate the use of the treatment methods proposed; patients with autoimmune diseases and on long term use of corticosteroids; pregnancy and lactation. Patients were asked about use of antibiotics during the study period and were aware that they could continue with the study; however, data after the time of antibiotics would not be included in the analysis. Third molars and dental implants were excluded from the analysis.

2.2.2.3 Exit Criteria

During the study period, subjects who developed any medical condition or systemic illness that contraindicated treatment or needed prophylactic antibiotics were to be exited from the study. All participants were informed that they were free to withdraw from the study at any time.

2.2.3 Clinical Study Design

The study design was a randomized controlled trial. Participants were allocated to test and control groups using consecutive allocation. The first patient that was screened, met the study requirements and consented to participate in the study was placed into the test group; the following patient screened, who met the inclusion criteria and consented to participate was allocated into the control group. This continued throughout the study and was not modified at any point of the study. To reduce allocation bias, participants in each group were informed of the treatment they were receiving but had no knowledge of the treatment options provided to participants in each group.

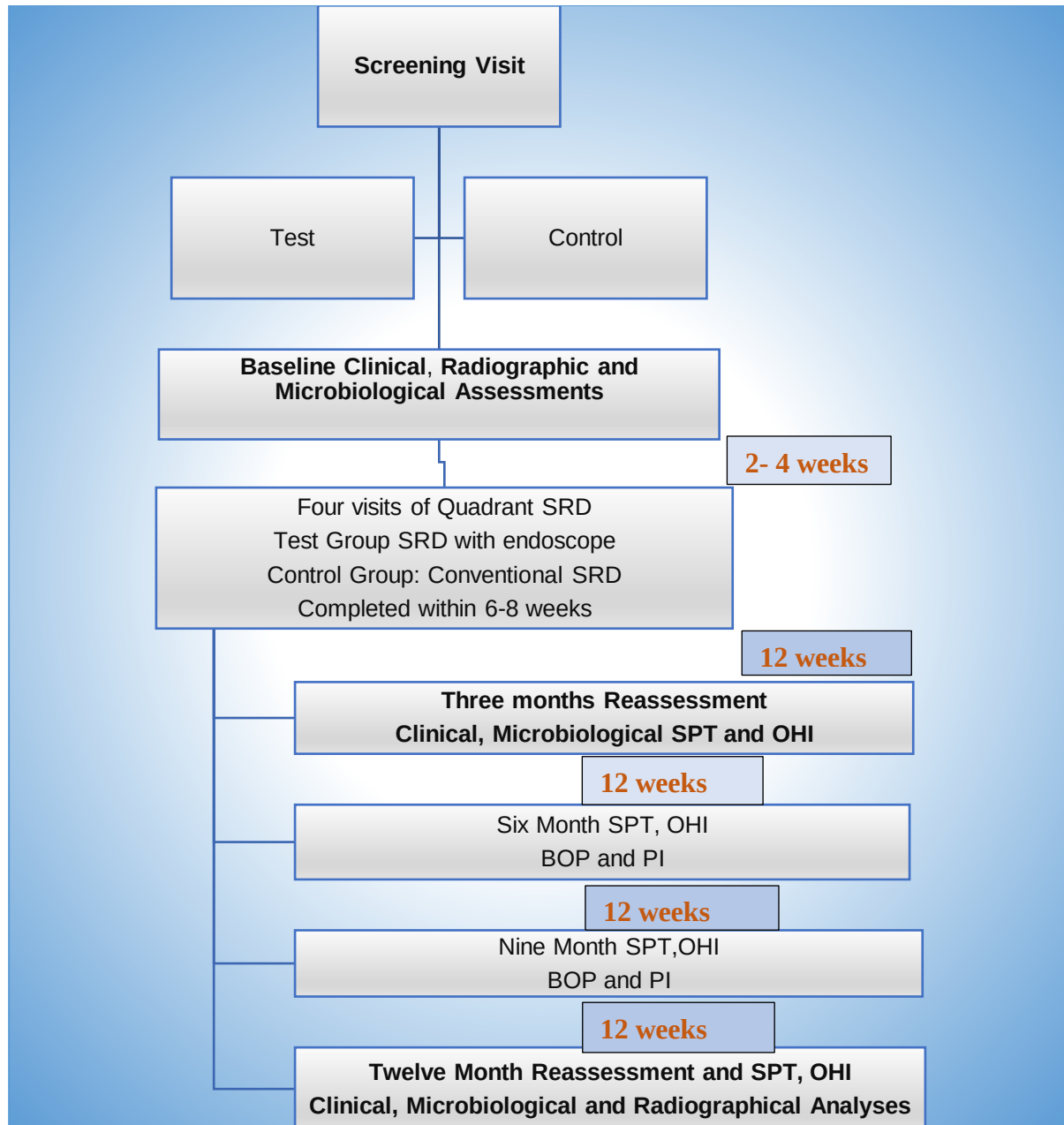
Baseline measurements, including clinical, microbiological and radiographical data were taken for all participants by MN prior to the commencement of non-surgical periodontal treatment. Baseline measurements included assessment of the gingival tissues including colour, consistency, contour and texture, PPDs, recession, PALs, BOP, PI, assessment of furcation areas, mobility, radiographic and microbiological analysis. Subjects in the test group were treated with SRD using the endoscope and participants in the control group with SRD alone. OHI were given according to individual needs. All treatment was completed by MN.

Figure 2.1. outlines the details of the treatment and assessments as well as time intervals between each visit.

After completion of the first phase of therapy, subjects in both groups were seen at the following visits.

- 1) three-month visit which included a comprehensive clinical and microbiological assessment and supportive periodontal maintenance and OHI,
- 2) six-month SPT, recording of BOP% and PI% and OHI,
- 3) nine-month supportive periodontal maintenance appointment, recording of BOP% and PI% and OHI,
- 4) twelve-month SPT, including a comprehensive clinical, radiographic and microbiological assessment and OHI.

Figure 2. 1 Study outline including details of treatment and assessments undertaken at each visit and time intervals between each visit



2.2.4 Clinical Examination

The following parameters were assessed and recorded.

2.2.4.1 Gingival Condition

The gingival colour, consistency of tissues, contour and texture were assessed and recorded prior to treatment and at each supportive periodontal maintenance appointment. The gingival colour was recorded as pale pink pigmented, red or bluish red. The consistency of gingival tissues was examined by pressing firmly on the gingival tissues and were recorded as either firm or spongy. The contour of the interdental papilla was documented as being knife edged, bulbous or rolled. The surface epithelium of the attached gingiva was assessed by drying the area with a piece of gauze and recording the texture as either smooth, shiny or stippled in appearance (Perry *et al.*, 1996). This was recorded at each visit; however, no statistical analyses were included to determine differences between groups at each time point due to the subjective nature of gingival assessment. It was merely used to educate patients at appointments and discuss areas of concern.

2.2.4.2 Probing Pocket Depths

PPDs were measured to the nearest millimeter from the gingival margin to the bottom of the gingival sulcus/pocket using a manual rigid periodontal probe (PCPUNC15, Hu Friedy, Chicago, IL, USA) (Lindhe *et al.*, 2008). Six recordings, mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual, were made for each tooth. The mean PPD (mm) was calculated for each group. PPD were also categorised as PPD 1-3 mm, 4-6 mm, 7-9 mm and 10+ mm and percentages for each category was calculated as described below.

2.2.4.2.1 Calculation of Average/Mean PPD

The average/mean PPD was calculated as follows for each participant:

Total number of sites was calculated by adding the number of teeth and then multiplying by six sites. The average PPD (mm) was calculated by summing the PPD of all sites divided by the total number of sites.

2.2.4.2.2 Calculation of Percentage of PPD For Each Category

The number of sites with PPDs in the categories, for example PPD 4-6 mm, were totalled. This total was then divided by the number of sites and multiplied by 100. This provided a percentage of sites with PPD in each category. This method was used for calculations at baseline, three and twelve months.

2.2.4.3 Recession

Gingival recession was measured clinically to the nearest millimetre from the CEJ to the free gingival margin in millimetres using the PCPUNC15 probe (Hu Friedy, Chicago, IL, USA) (Wilson *et al.*, 1996). Mid facial, mid-palatal as well as interproximal recessions were recorded for each tooth. Gingival enlargement or hyperplasia was recorded as a negative value. The mean recession was calculated by totalling the recession for each site and then dividing by the number of sites.

2.2.4.4 Probing Attachment Levels

The PAL was calculated using the PANDA (©Panda Dental Software, Inc, Bellevue, WA, USA) periodontal software system by adding the gingival recession and PPD for each area. Six recordings including mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual were made for each tooth. The mean PAL (mm) was calculated for each group.

PAL were also categorised as PAL 1-3 mm, 4-6 mm, 7-9 mm and 10+ mm and percentages were calculated for each category as described below.

2.2.4.4.1 Calculation of Average/Mean PAL

The average/mean PAL was calculated as follows:

The total number of sites was calculated by adding the number of teeth and then multiplying by six sites. The average PAL (mm) was calculated by summing the PAL of all sites divided by the total number of sites.

2.2.4.4.2 Calculation of Percentage of PAL for Each Category

The total number of sites with PAL in each category was calculated. This total was then divided by the number of sites and multiplied by 100. This provided the percentage (%) of sites with PAL in each category. This method was used for calculations at baseline, three and twelve months.

2.2.4.5 Assessment of Furcation Involvement

The Nabers furcation probe (Hu Friedy, Chicago, IL, USA) graded at 3 mm was utilised to assess the extent of furcation involvement on multi-rooted teeth. Furcation involvement was measured from all entrances of multi-rooted teeth including the buccal and lingual of mandibular molars. Maxillary premolars and molars were assessed from the buccal, disto-palatal and mesio-palatal aspects. The furcation between the mesio-buccal and palatal roots were measured from the palatal aspect. Furcation involvement was classified as follows:

Class I – Horizontal probing ≤ 3 mm from one or two entrances.

Class II – Horizontal probing greater than 3 mm in one furcation entrance or in combination with a degree 1

Class III – Horizontal probing greater than 3 mm in two or more entrances

(Hamp *et al.*, 1975)

2.2.4.6 Tooth Mobility

Tooth mobility was measured by using two handles of two instruments positioned buccally and lingually (Noble, 2012). The Miller (1950) classification was used to classify tooth mobility

Degree 0 – “physiological” mobility – 0.1 mm -0.2 mm in a horizontal direction

Degree 1 – increased mobility of the crown up to 1 mm in a horizontal direction

Degree 2 – visual increased mobility of crown greater than 1 mm in a horizontal direction

Degree 3 – severe mobility of the crown both in a horizontal and vertical direction

2.2.4.7 Bleeding on Probing

The Ainamo & Bay index (1975) was used to assess BOP. Six sites per tooth were assessed including the mesio-buccal, buccal, disto-buccal, mesio-palatal/lingual, palatal/lingual and disto-palatal/lingual. If bleeding occurred within 30 seconds after periodontal probing, a positive finding was recorded (Ainamo *et al.*, 1975).

The percentage of sites with BOP was calculated as follows:

Number of sites with BOP / total number of sites assessed x 100

BOP % was calculated at baseline, three, six, nine and twelve-month appointments.

2.2.4.8 Plaque Index

The PI was calculated as a percentage using the O'Leary, Drake and Naylor PI (O'Leary *et al.*, 1972). Disclosing solution was applied to tooth surfaces to visualise plaque. The presence or absence of plaque on the mesial, distal, facial and lingual surfaces on each tooth was recorded.

The number of sites with plaque divided by the total number sites multiplied by 100 provided a percentage of plaque at each visit. This index was used to motivate and instruct patients on proper oral hygiene techniques. PI% was calculated at baseline, three, six, nine and twelve-month appointments.

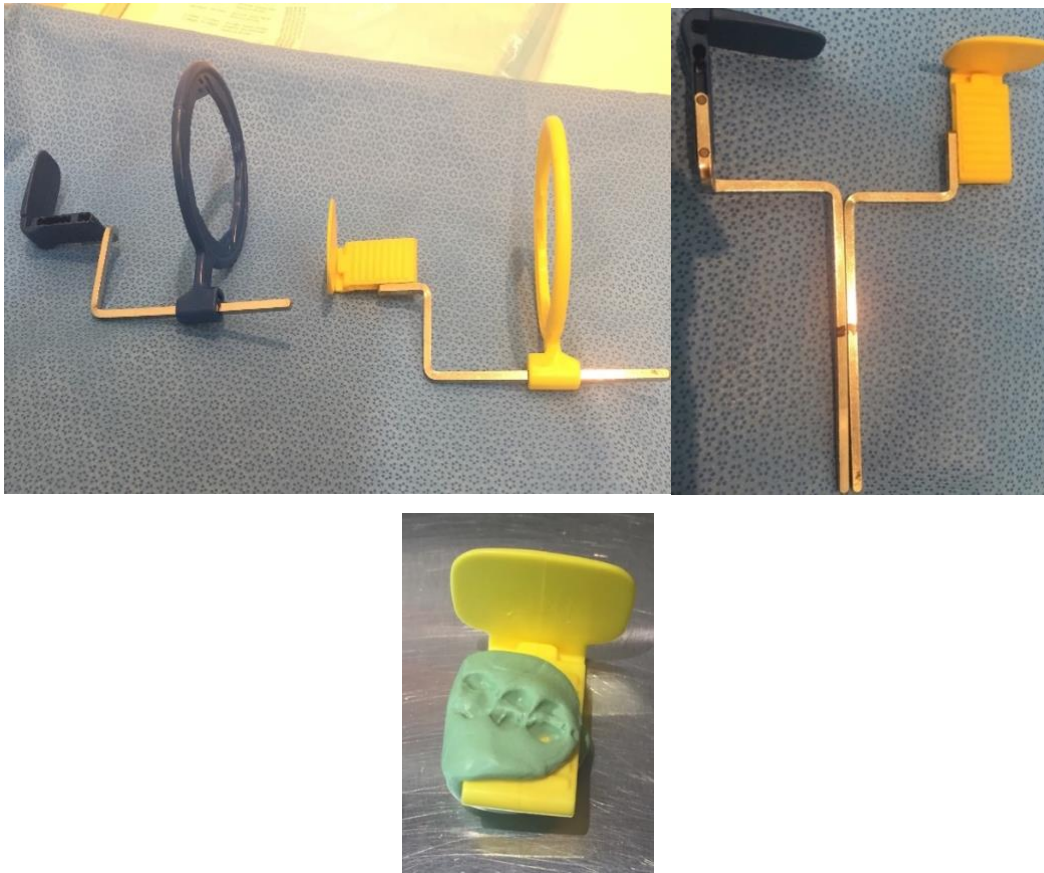
2.2.4.9 Consistency of Clinical Data

One examiner (MN) was responsible for all clinical data collection as well as all treatment performed on study participants. The examiner is a qualified dental hygienist, with 25 years' experience in dental hygiene practice. Clinical records taken at each point of treatment were checked by AR (Periodontist) to ensure consistency and accuracy of clinical records. There was no disagreement of more than 1 mm observed.

2.2.4.10 Radiographic Analyses

Standardised radiographs were taken for both test and control groups. Radiographs were taken at sites with the deepest pockets and in sites displaying angular/vertical bone loss. Radiographs were standardised using the Rinn XCP holding devices. Digital radiographic sensors were placed parallel to the area of interest with the locating ring placed at a fixed distance from the sensor. Silicone rubber impression material was used to create a positioning stent for each area radiographed (Figure 2.2.). The material was mixed as per the instructions provided by the manufacturer and securely placed and sealed on the bite block using an adhesive. Patients were then asked to bite on the material, thereby creating a positioning stent of each area being radiographed. These stents were carefully labelled and stored and were used again at the 12-month re-evaluation SPT appointment. At this appointment, patients were asked to bite so that the upper and lower teeth fit into the customised positioning stent.

Figure 2. 2 Standardisation of radiographs using Rinn XCP holders and positioning stents



2.2.4.10.1 Calculating Radiographic Bone Levels Using the Digimizer Image Analysis Software.

The standardised views taken before the commencement of treatment were compared to the radiographs taken at the 12-month appointment using the Digimizer Image Analysis software (©2005=2018 MedCalc Software bvba, Belgium).

This software system allows manual measurements to be taken and measures distances and lengths of segments. In order to standardise measurements taken, the dimensions of the radiographic sensors were measured and included. The dimensions of the sensor were obtained by measuring the actual size of the radiographic sensor

(size 1 and 2 sensors). Size 2 sensors were 40 mm and Size 1 sensors 23 mm. These dimensions were set as the default calibration in the software.

The distance from the CEJ to the bone crest was measured for each area visible on the radiograph. This represented the RBL (Figure 2.3.)

Figure 2. 3 Radiographic film with analysis using Digimizer Image Analysis software



Teeth were also categorised as single-rooted and multi-rooted teeth and changes in mean RBL were also measured.

The number and percentage of sites displaying vertical and horizontal bone loss for the test and control group were calculated. If the crest of the alveolar bone appeared to be parallel to the line drawn between the CEJ of adjacent teeth, reduction in height of the interdental septum was considered as horizontal bone loss. Bone loss occurring in an angular or oblique direction was considered as vertical bone loss. Luan Ngo (LN) and MN both assessed areas on radiographs and agreed on areas of vertical and horizontal bone loss.

Radiographic bone loss, bone gain and no changes in RBLs were calculated for each area by subtracting the mean RBL pre-treatment from the mean RBL measured at

twelve months. A positive reading (+) mm indicated bone gain, a negative reading (-) mm indicated bone loss and a reading of (0) mm indicated no change in RBL.

2.2.4.10.2 Operator Calibration (Radiographic Analysis)

Operator calibration and statistical analysis to describe agreement between measurements is described below.

2.2.4.10.3 Accuracy of Radiographic Measurements.

One examiner (MN) was responsible for the measurement of RBLs for both groups. Measurements were repeated for sites to ensure accuracy.

Figure 2.4 depicts the agreement between the measurements. To formally assess the agreement the Bland-Altman method was used. From Figure 2.5 it can be seen that the mean difference is 0.00. The limits of agreement (ULA and LLA) as shown on the Bland Altman plot indicate that the majority of the measurements differ by less than 0.03. The largest difference is 0.06. Given the measurement scale, this is an excellent agreement. Table 2.1 displays the estimation for paired difference. This indicates that on average the measurements are virtually identical.

Figure 2. 4 Agreement between measurements

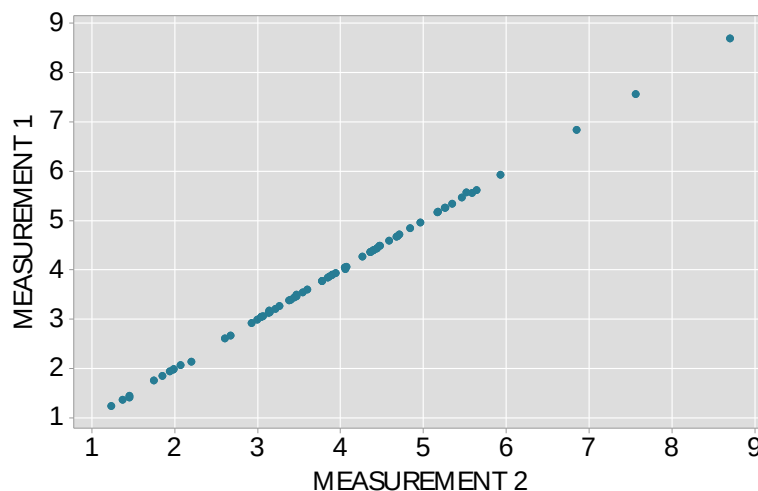


Figure 2. 5 Bland-Altman Plot

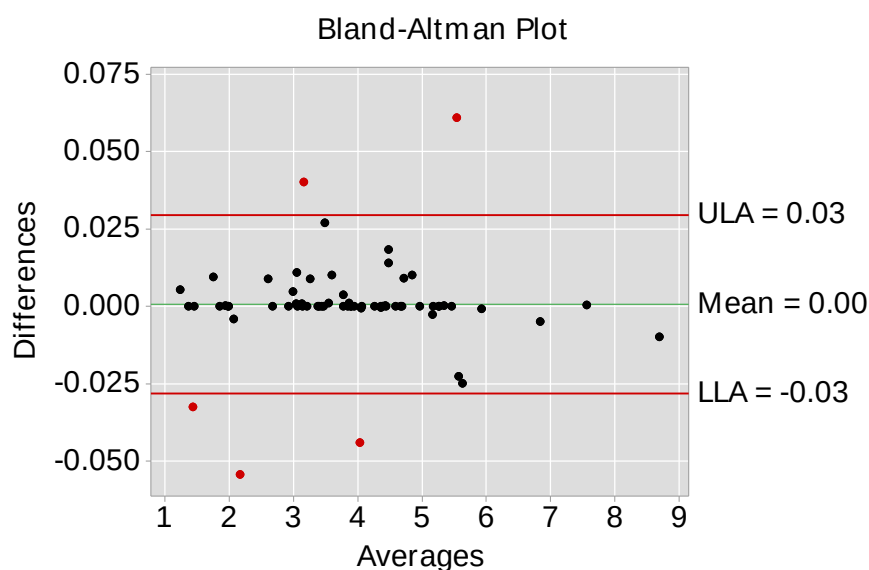


Table 2. 1 Estimation for Paired Difference

Mean	Std Deviation	SE of Mean	95% CI for $\mu_{\text{difference}}$ *
0.00062	0.01471	0.00178	(-0.00294, 0.00418)

* $\mu_{\text{difference}}$: mean of (MEASUREMENT 1 - MEASUREMENT 2)

2.3 Microbiological Testing Protocol

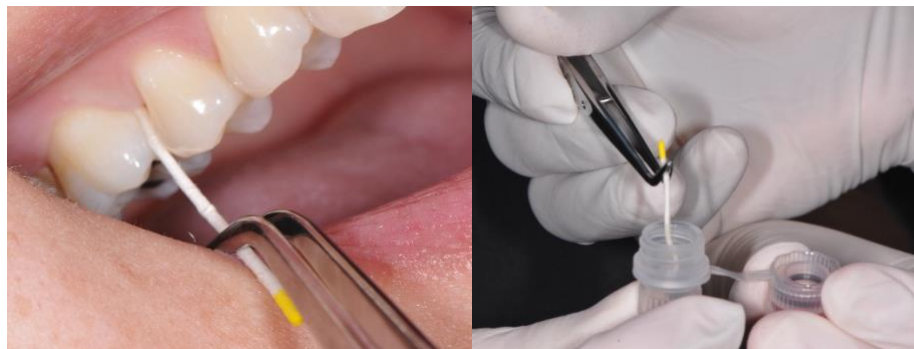
The Hain Lifescience Micro-IDent Plus system (Nehren, Germany) was used to collect plaque samples of all participants. This test provides information on the levels of the eleven putative pathogens and their affiliation to the “bacterial complexes”. The bacterial sampling pack contains five sterile paper points in a sterile sleeve, a sterile test tube for collection of the samples as well as a water-resistant bag to securely seal and store samples (Figure 2.6).

Figure 2. 6 Micro-IDent plus Kit with sterile paper points and test tube



The areas to be sampled were dried with sterile gauze prior to insertion of the sterile paper points. The manufacturer's instructions were followed for sampling. The paper points were removed from the sterile sleeve with sterile tweezers and then one immediately inserted into each of the five of the deepest periodontal pockets for 10 seconds. Paper points are then placed into the sterile test tube provided (Figure 2.7).

Figure 2. 7 Sterile paper point inserted in periodontal pocket and placed in test tube



Test tubes containing the paper points were left open and allowed to dry to one hour before sealing the test tube and placing in secure water-resistant bags provided by the company (Figure 2.8).

Figure 2. 8 Storage and postage



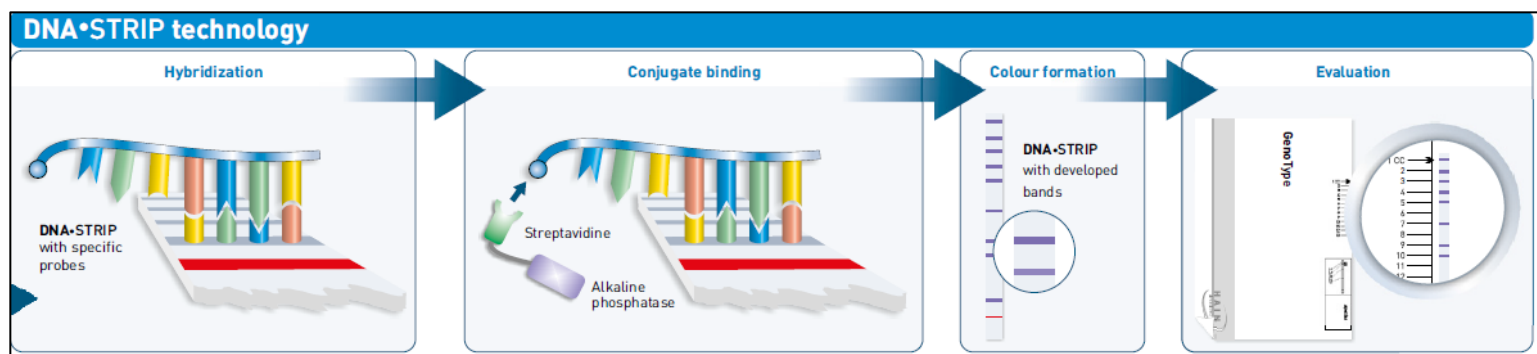
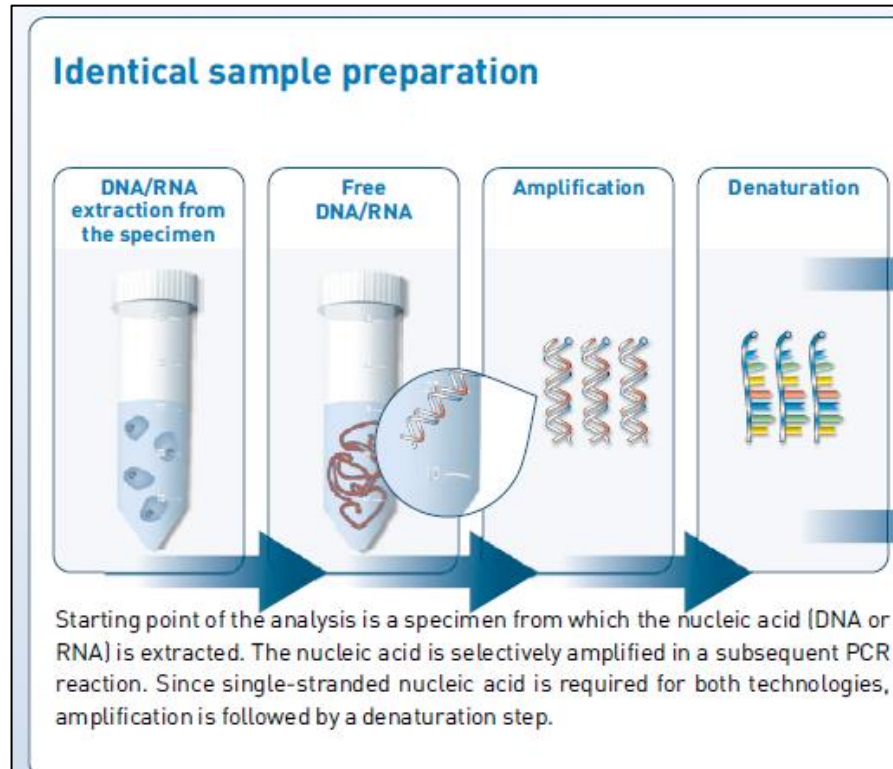
Samples were stored in the freezer (-20°C). When enough samples were collected, they were posted via FedEx to Hain Lifescience laboratory in Nehren, Germany.

Plaque samples were taken before the commencement of SRD; at the three-month post-SRD and at the twelve-month appointment. Sample sites remained the same at each point so as to compare the effects of non-surgical therapy with or without the use of the perioscope.

2.3.1 Mechanism of Analyses

Eleven putative pathogens, namely *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia*, *T. denticola*, *P. micro*, *F. nucleatum*, *C. rectus*, *E. nodatum*, *E. corrodens* and *Capnocytophaga sp.*, respectively were analysed. The Micro-IDent Plus test utilizes two distinct techniques, namely PCR and reverse hybridization (Eick *et al.*, 2011). DNA membrane strip technology is used. The DNA or RNA is isolated from specimens, amplified and identified via hybridization and alkaline phosphatase reaction. Following isolation, the nucleic acids are selectively replicated. The DNA strips are covered with probes that are highly specific and correspond to selectively amplified nucleic acid sequences. During the hybridization process, the single-stranded amplicon binds to the analogue probes while the non-specifically bound amplicons are removed in the washing steps. The specifically bound amplicon is marked with enzyme alkaline phosphatase and is made visible in a colorimetric detection reaction. A banding pattern develops on the DNA strip. A test-specific template is used making it quick and easy to interpret (Urban *et al.*, 2010) (Figure 2.9).

Figure 2. 9 Sample Preparation and DNA Strip Technology (taken from Hain Lifescience information booklet)



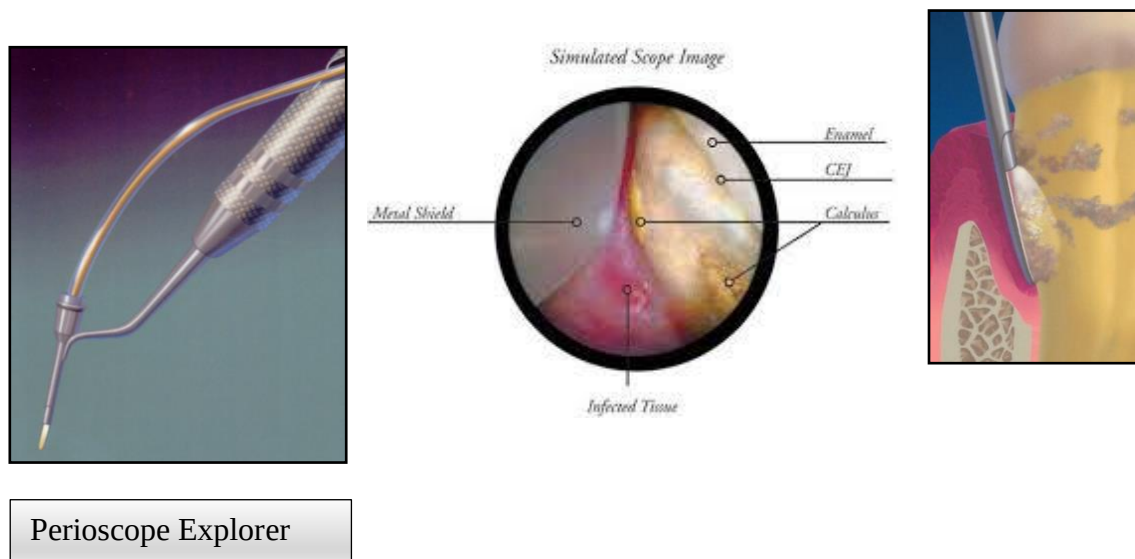
2.4 Non-Surgical Periodontal Therapy

Participants in the test group were treated with SRD with the periodontal endoscope whilst those participants in the control group were treated using SRD alone. All treatment was completed utilizing topical and local anaesthetic (Hurricane Topical Anaesthetic 20% Benzocaine; Scandonest 3% (mepivacaine hydrochloride 66mg) block; Septanest 2% (articaine hydrochloride 88mg and adrenaline 22 ug for infiltration). Treatment involved quadrant debridement with 1.5 hours allocated for debridement for each quadrant. To maintain consistency, all hand and powered instruments used for both test and control groups were the same. Hand instruments included the Gracey area specific after 5 curettes, (posterior and anterior) and the universal 4R/L and 2R/2L curettes (Hu Friedy, Chicago, IL, USA). Powered instruments included the 30K slimline cavitron insert by Dentsply (Dentsply Sirona, USA). The only difference between the test and control groups was the use of the endoscope in the test group.

2.4.1 Endoscope Assisted Scaling and Root Debridement

A camera attached to a tiny probe was inserted below the gum line using a perioscope explorer (Zest Dental Solutions, USA). Four explorers (two straight left and right, two curved left and right) were used to access surfaces of the teeth. The magnified root surface (magnification varied between 24x to 46x) was visible about 3 mm at a time on the screen/monitor. The magnification depended on the distance of the object from the tip of the endoscope. The subgingival area including the inflamed pocket, subgingival calculus and biofilm deposits were visualized. Both power and hand instruments were used to remove all visible calcified deposits from the root surface (Stambaugh *et al.*, 2002; Wilson *et al.*, 2008a). (Figure 2.10)

Figure 2. 10 Perioscope technology



2.5 Supportive Periodontal Therapy

Subjects were recalled at three-month intervals after completion of non-surgical periodontal therapy. The SPT included recording of plaque and bleeding indices; reinforcement of home care instructions where required; as well as the removal of supra and subgingival plaque and calculus deposits. This included the use of hand scalers, curettes and ultrasonic instruments. Topical and local anaesthetic (Hurricane Topical Anaesthetic 20% Benzocaine; Scandonest 3% (mepivacaine hydrochloride 66mg) block; Septanest 2% (articaine hydrochloride 88mg and adrenaline 22 ug for infiltration) were used if patients reported sensitivity in areas. At the end of the clinical trial (twelve-month SPT appointment), all participants were assessed by AR and given recommendations for further treatment, which included continuing with SPT, periodontal surgery, perioscopy or any other specialist work required for example dental implants or orthodontics.

2.6 Statistical Analyses of Data

Clinical, microbiological and radiographical data were analysed using IBM SPSS Statistics Version 25 Statistical software (SPSS Inc. Chicago, IL, USA) and Minitab statistical package (Minitab®18, Minitab Inc, State College, PA). All thirty-eight participants were included in the analyses and there were no exclusions at three and twelve months. A statistician (SF) from the Statistical Consulting Centre, University of Melbourne assisted with data analysis.

2.6.1 Statistical Analysis of Clinical Data

Clinical data including PPD, PAL, the respective categories and gingival recession were analysed for differences in means between the test group and the control group at baseline, three months and twelve months. For BOP and PI, statistical analysis was carried out for baseline, three, six, nine and twelve months.

The independent samples T-test was used to test for statistically significant differences between means for the two groups for all clinical parameters. Descriptive statistics including the mean, standard deviation and standard error of mean were reported for each group. Confidence intervals are also described. $P < 0.05$ was considered as statistically significant. Mean and confidence intervals were represented graphically with individual standard deviations used to calculate the intervals.

Change in mean gingival recession was also calculated from baseline to twelve months for both groups. Independent samples T-tests were used to test for statistically significant differences between groups for change in mean gingival recession (mm).

2.6.2 Statistical Analysis of Radiographic Data

The number of radiographs taken for each group prior to commencement of treatment and at twelve months were calculated. Also included are the number of radiographs that were suitable for analysis. Measurements taken before commencement of treatment were compared to measurements taken at the twelve-month appointment.

The mean/average RBL (mm) were calculated by adding the RBLs measurement of all sites and then dividing by the number of sites. The mean was calculated for pre-treatment radiographs and radiographs taken at twelve months for each group. The mean difference in RBL (RBL) mm for each site was calculated as follows:

$$\text{RBL (Pre-SRD) minus RBL (Twelve Months)} = \text{mean change in RBL (mm)}$$

Teeth were also divided into single and multi-rooted teeth and analysed. Mean differences in RBLs for single and multi-rooted teeth were calculated in the same way as described above.

Sites were assessed to determine the type of bone loss, that is either horizontal or vertical/angular bone loss. The number and percentage of sites displaying horizontal and vertical bone loss were calculated for both groups.

The percentage of sites with radiographic bone gain, bone loss and no change in RBLs were also calculated for sites displaying vertical/angular and horizontal bone loss. This was calculated as follows:

Number of sites with Radiographic Bone Gain/Loss/No change divided by the total number of surfaces multiplied by 100.

This same method was used for single and multi-rooted teeth.

The independent samples T-test was used to test for a statistically significant differences between mean RBL (mm) for the test and control groups. The mean, standard deviation and standard error of mean were reported for each group. $P < 0.05$ was considered as statistically significant. Mean and confidence intervals were represented graphically with individual standard deviations used to calculate the intervals. The minimum, maximum and range in millimetres were also computed. Range of values of RBL in 0.5 mm increments were calculated and frequency and range were represented graphically and compared between the test and control groups.

2.6.3 Statistical Analysis of Microbiological Data

Three reports were received from Hain Lifescience for each participant. This included a pre-SRD report, post- SRD report and a report at twelve months. The data received from Hain Lifescience is as below: (Figure 2.11)

(-) Bacterial load under cut off = $<10^4$ with the Exception of

A. actinomycetemcomitans $<10^3$

(+) Bacterial load at cut off = 10^4 with the Exception of

A. actinomycetemcomitans: 10^3

+ Increased Bacterial load = $<10^5$ with the Exception of

A. actinomycetemcomitans $<10^4$)

++ High bacterial load = $<10^6$ with the Exception of

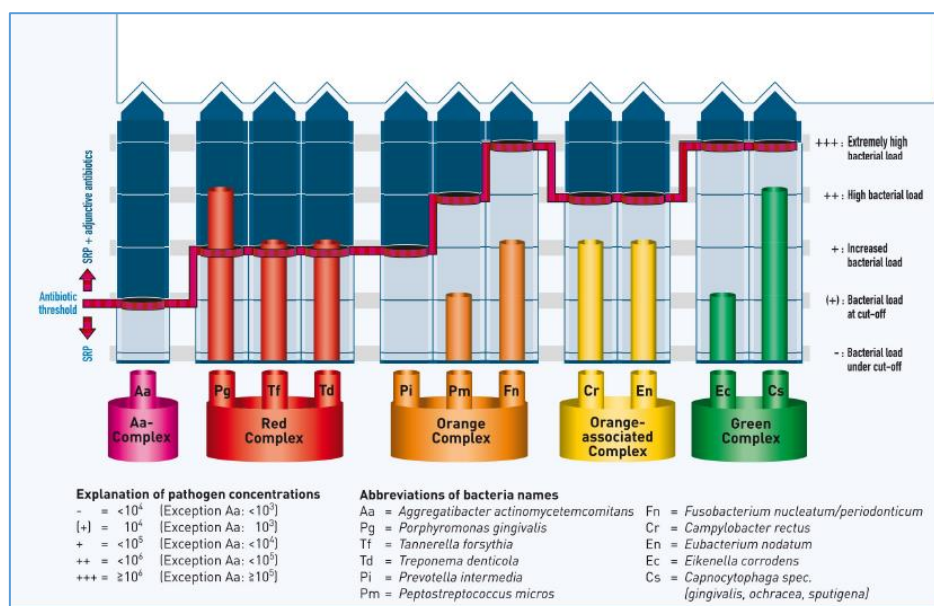
A. actinomycetemcomitans $<10^5$

+++ Extremely high bacterial load = $>10^6$ with the Exception of

A. actinomycetemcomitans $> 10^5$

Suggestions regarding antibiotic therapy were also provided by the company; however, this was not implemented during the treatment or SPT phase of therapy.

Figure 2. 11 Example of results received from Hain Lifescience



For purposes of statistical analyses, the data received for the eleven pathogens were ranked as follows:

Level 0: (-) Bacterial load under cut off = <10⁴ with the Exception of *A. actinomycetemcomitans* <10³

Level 1: (+) Bacterial load at cut off = 10⁴ with the Exception of *A. actinomycetemcomitans*: 10³

Level 2: + Increased Bacterial load = <10⁵ with the Exception of *A. actinomycetemcomitans* <10⁴

Level 3: ++ High bacterial load = <10⁶ with the Exception of *A. actinomycetemcomitans* <10⁵

Level 4: +++ Extremely high bacterial load = >10⁶ with the Exception of *A. actinomycetemcomitans* > 10⁵

Data received from the company also included the bacteria in their complexes.

In order to analyse the bacteria in their complexes, **the total rank** was calculated with the maximum for a rank being 4. If there were three pathogens in the complex, the maximum would be 12. If there were only two pathogens, the maximum for the complex would be 8. Using these maximum values, the complexes were ranked as follows:

Red and Orange Complex

- Level 0: (-) Bacterial Load below cut off (0;1)
- Level 1: (+) Bacterial load at cut off (2;3;4)
- Level 2: (+) Increased bacterial Load (5;6;7)
- Level 3: (++) High Bacterial Load (8;9;10)
- Level 4: (+++) Extremely high bacterial load (11;12)

For example, if in the red complex, the total for the three pathogens were 7, they were ranked as “2” (+Increased bacterial load); if the total was 9, the complex was ranked as 3 (++ High bacterial Load).

Orange Associated and Green Complex

- Level 0: (-) Bacterial load under cut off (0;1)
- Level 1: (+) Bacterial load at cut off (2;3)
- Level 2: (+) Increased bacterial load (3;4)
- Level 3: (++) High bacterial Load (5;6)
- Level 4: (+++) Extremely high bacterial load (7;8)

2.6.3.1 Statistical Analysis of Individual Pathogens

The Mann-Whitney *U* test was used to compare the two independent samples of ordinal(ranked) data for individual bacteria. The mean rank was reported. The mean rank was obtained by ordering the entire data set. Once a rank had been assigned to each case, they were returned to their respective groups (namely control and test groups) and then summed and averaged. The mean rank were these averages. The mean ranks allowed comparison of the ranks between the two groups for each pathogen, similar to the T-test. The *U* statistic indicates the greatest possible difference between the test and control groups. $P < 0.05$ was considered statistically significant. The mean rank, *U*, *z* and *p* value were included for each of the eleven pathogens at the different time points and compared between the study groups. The percentages for each pathogen for each level was also described and compared between the test and control groups at each time point and represented graphically.

Prevalence of each pathogen at below detectable levels (level 0) and above detectable levels (level 1,2,3,4) were compared at each time point. Levels were grouped into “below threshold” and “above threshold”. Percentage of each pathogen at below and above threshold levels were computed for both control and test groups pre-SRD, post-SRD and at twelve months.

2.6.3.2 Statistical Analysis of Bacterial Complexes

The bacterial complexes were analysed and reported in exactly the same way as for individual pathogens using the Mann-Whitney U tests. The percentages of each complex were calculated for each level and graphically represented.

CHAPTER 3

PRE- AND POST-TREATMENT CLINICAL RESULTS AND SUPPORTIVE PERIODONTAL MAINTENANCE

This chapter compares pre- and post-treatment clinical parameters between SRD alone and SRD with the aid of the perioscope. Clinical measurements including PPDs, PALs and gingival recession were compared at pre-SRD, post-SRD and twelve months. Patients were recalled at three-month intervals for supportive periodontal therapy. BOP and plaque scores were documented at all SPT visits and analysed at each of the time points. The average time between SPT appointments is included and analysed.

The demographics of the study population, their smoking status and medical status are described. The effect of the SRD-alone is compared to SRD-with perioscope using independent sample T-tests and comparing means between groups. Confidence intervals are diagrammatically represented.

3.1 Demographics of The Study Population

Fifty-seven subjects were approached, of which thirty-nine consented to be included in the study. All thirty-nine patients completed initial phase therapy, however one patient failed to attend the re-evaluation appointment at three months and was excluded from the study. Hence, thirty-eight subjects were included in the statistical analysis (19 in the control group; 19 in the test group) at three, six, nine and twelve months. Figure 3.1 represents a consort flow diagram as patients went through enrolment, allocation and follow up

The mean age of participants was 52.50 ± 11.05 years (range 28-75 years). There were 24 females and 14 males (Table 3.1). The majority of the subjects were non-smokers (84.2%), 7.9% were smokers (5.3% in the control group and 10.5% in the test group); 7.9% were past smokers (10.5% in the control group and 5.3% in the test group) (Table 3.2).

Twenty three of the thirty-eight patients had no relevant medical history (NRMH) (Table 3.3). Two patients were Type II diabetics but controlled with medication. One patient had fibromyalgia but did not take any medications and was therefore included in the study. There were no statistically significant differences between groups with regards to age, gender, smoking status and medical history.

The average time between three monthly SPT was 90.5 ± 4.5 days in the control group and 91.08 ± 2.2 days in the test group. The difference in average time in days between the two groups were not statistically significant ($p=0.6$) (Table 3.1).

Figure 3. 1 Consort Flow Diagram

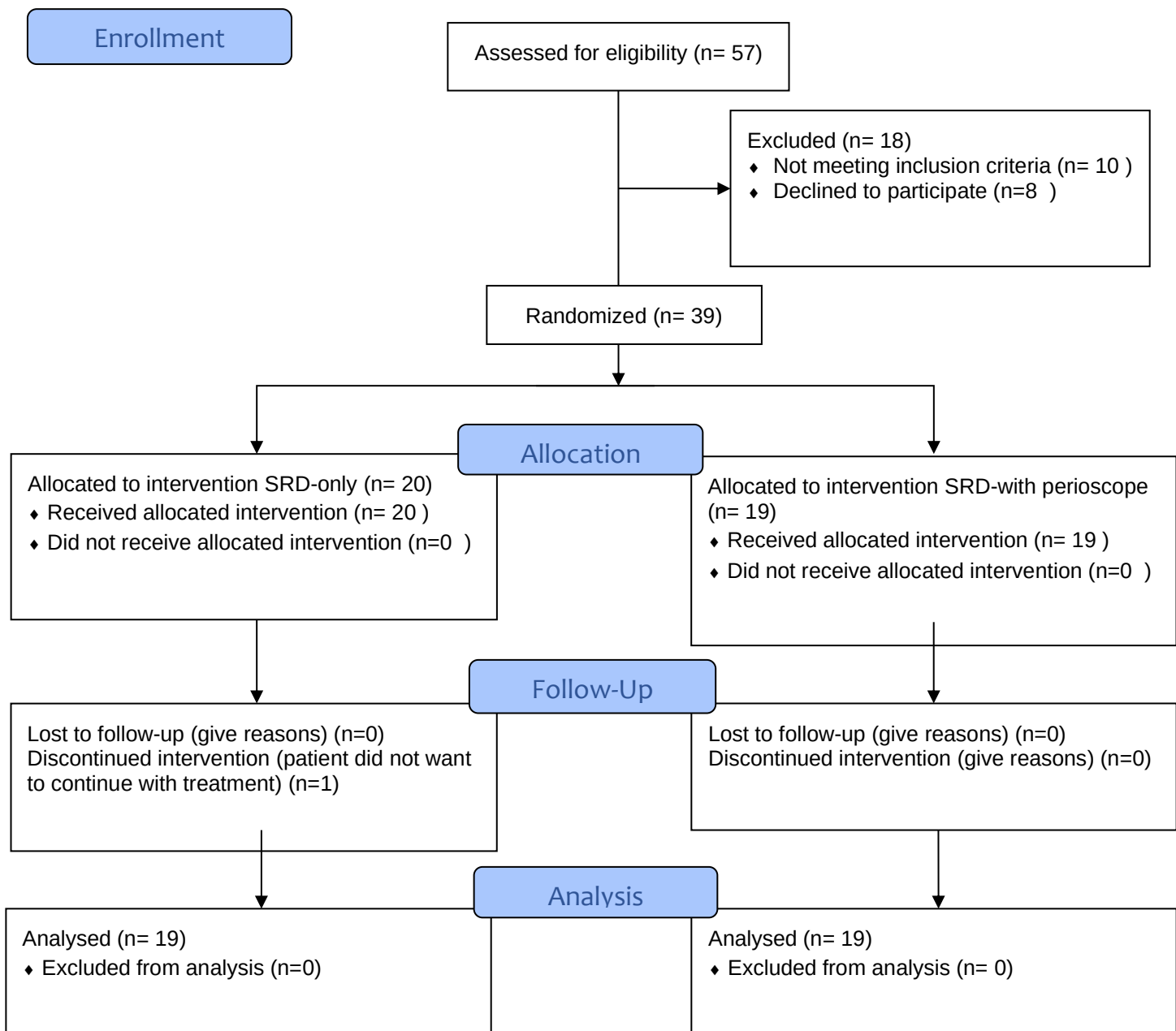


Table 3. 1 Demographics of study population

	Control (n=19)	Test (n=19)	
Male	6 (31.6%)	8 (42.1%)	14 (36.8%)
Female	13 (68.4%)	11 (57.9%)	24 (63.2%)
Mean Age (SD) and Range in years	51.37(±13.04) 28-47 years	53.63(±8.28) 38-68 years	52.50 (±11.05) 28-75 years
Smokers	5.3%	10.5%	7.9%
Average time between SPT appointments in days	90.49(±4.5)	91.08(±2.2)	p=0.6

Table 3. 2 Smoking status of the study population

Smoking status	Control (n=19)	Test (n=19)	Total (n=38)
Non-smokers	16(84.2%)	16(84.2%)	32(84.2%)
Past Smokers	2(10.2%)	1(5.3%)	3(7.9%)
Smokers	1(5.3%)	2(10.2%)	3(7.9%)
Total	19(100%)	19(100%)	38(100%)

Table 3. 3 Medical history of the study population

Medical history	Control (n=19)	Test (n=19)	Total (n=38)
NRMH	13(68.4%)	10(52.6%)	23(60.5%)
High blood pressure	1(5.3%)	1(5.3%)	2(5.3%)
Asthma	1(5.3%)	1(5.3%)	2(5.3%)
Arthritis	1(5.3%)	0(0%)	1(2.6%)
High cholesterol	0(0%)	1(5.3%)	1(2.6%)
Allergy to penicillin	1(5.3%)	2(10.5%)	3(7.9%)
Allergy to erythromycin	0(0%)	1(5.3%)	1(2.6%)
Fibromyalgia	1(5.3%)	0(0%)	1(2.6%)
High blood pressure and high cholesterol	1(5.3%)	0(0%)	1(2.6%)
Diabetes and high cholesterol	0(0%)	1(5.3%)	1(2.6%)
Osteoporosis and liver disease	0(0%)	1(5.3%)	1(2.6%)
High blood pressure and diabetes	0(0%)	1(5.3%)	1(2.6%)
Total	19(100%)	19(100%)	38(100%)

3.2 Effect on SRD-alone and SRD-with perioscope on clinical parameters

The baseline, three month and twelve-month clinical parameters including PPD, PAL, and mean recession is presented in Table 3.4. PPDs were categorized as PPDs 1-3 mm; 4-6 mm; 7-9 mm; 10 mm and above. PAL's were categorized as PAL 1-3 mm; 4-6 mm; 7-9 mm; 10 mm and above. Percentages of PPD and PAL were calculated in the categories and compared between the test and control groups.

Table 3.5 illustrates the mean percentage of BOP at baseline, three, six, nine and twelve months

Table 3. 4 Summary of statistics for mean PPD, PPDs 1-4 mm, 5-7 mm, 8-10 mm and 10+ mm, mean PAL, PALs 1-3 mm, 4-6 mm, 7-9 mm, 10+ mm and mean recession at baseline, three and twelve months for control and test groups

	Control (n=19) SRD - only		Test (n=19) SRD- with perioscope		Independent sample t-test summary statistics	
Variable	Mean, standard deviation	Standard error of mean	Mean, standard deviation	Standard error of mean	95% CI	p-value
Mean PPD (mm)						
Baseline	4.63 ±0.8	0.19	4.24 ±0.6	0.13	-0.8;0.9	0.1
Three months	3.05 ±0.5	0.12	2.89 ±0.3	0.06	-0.1;0.4	0.2
Twelve months	2.98 ±0.4	0.10	2.70 ±0.2	0.05	0.0;0.5	0.02
PPD 1-3 mm %						
Baseline	30.14 ±17.9	4.11	39.21 ±17.7	4.07	-20.8;2.7	0.1
Three months	79.28 ±14.8	3.40	82.88 ±7.5	1.71	-11.3;4.2	0.4
Twelve months	81.59 ±13.0	2.98	86.66 ±6.3	1.44	-11.8;1.8	0.1
PPD 4-6 mm %						
Baseline	57.22 ±14.0	3.22	54.06 ±16.0	3.68	-6.7;13.1	0.5
Three Months	18.23 ±12.5	2.88	16.42 ±7.1	1.62	-4.9;8.6	0.6
Twelve Months	16.32 ±11.4	2.61	12.84 ±5.9	1.34	-2.5;9.5	0.2
PPD 7-9 mm %						
Baseline	11.56 ±12.6	2.88	6.32 ±4.6	1.05	-1.0;11.6	0.1
Three months	2.25 ±2.9	0.66	0.70 ±1.2	0.27	0.1;3.0	0.04
Twelve months	1.84 ±2.3	0.54	0.50 ±1.0	0.23	0.2;2.6	0.03

PPD 10+ mm %						
Baseline	1.07 ±1.8	0.41	0.41 ±1.0	0.24	-0.3;1.6	0.2
Three months	0.23 ±0.8	0.18	0.00 ±0.0	0.00	-0.1;0.6	0.2
Twelve months	0.23 ±0.8	0.17	0.00 ±0.0	0.00	-0.1;0.6	0.2
Mean PAL (mm)						
Baseline	5.09 ±0.8	0.19	5.13 ±0.6	0.15	-0.5;0.4	0.9
Three months	3.96 ±0.7	0.72	3.93 ±0.5	0.48	-0.4;0.4	0.9
Twelve months	3.94 ±0.7	0.16	3.73 ±0.5	0.11	-0.2;0.6	0.3
PAL 1-3 mm %						
Baseline	19.05 ±14.9	3.42	16.99 ±11.2	2.56	-6.6;10.8	0.6
Three months	47.26 ±17.2	3.96	44.52 ±16.8	3.85	-8.5;13.9	0.6
Twelve months	47.78 ±17.8	4.09	48.81 ±16.3	3.74	-12.3;10.2	0.9
PAL 4-6 mm %						
Baseline	60.53 ±12.8	2.93	63.83 ±10.3	2.37	-10.9;4.3	0.4
Three months	44.82 ±12.2	2.81	50.08 ±15.4	3.53	-14.4;3.9	0.3
Twelve months	44.33 ±12.9	2.95	47.42 ±15.2	3.49	-12.4;6.2	0.5
PAL 7-9 mm %						
Baseline	18.20 ±12.3	2.83	16.78 ±8.9	2.04	-5.6;8.5	0.7
Three months	7.05 ±8.3	1.91	5.13 ±4.1	0.94	-2.4;6.3	0.4
Twelve months	6.96 ±7.4	1.70	3.53 ±3.2	0.73	-0.3;7.2	0.07
PAL 10+ mm %						
Baseline	2.21 ±3.2	0.72	2.40 ±2.8	0.63	-2.1;1.8	0.9
Three months	0.86 ±1.4	0.31	0.26 ±0.4	0.09	-0.1;1.3	0.08
Twelve months	0.94 ±1.5	0.34	0.23 ±0.3	0.07	-0.0;1.4	0.05
Mean recession (mm)						
Baseline	0.46 ±0.7	0.15	0.90 ±0.4	0.10	-0.8; -0.1	0.03
Three months	0.91 ±0.4	0.10	1.0 ±0.4	0.09	-0.4;0.1	0.3
Twelve months	0.96 ±0.4	0.10	1.03 ±0.4	0.09	-0.3;0.2	0.5

Table 3. 5 Summary of statistics for BOP% and PI% at baseline, three, six, nine and twelve months for control and test groups

	Control (n=19) SRD - only		Test (n=19) SRD- with perioscope		Independent sample t-test summary statistics	
Variable	Mean, standard deviation	Standard error of mean	Mean, standard deviation	Standard error of mean	95% CI	p-value
BOP %						
Baseline	60.16 ±19.6	4.50	48.37 ±16.8	3.86	-0.2;23.8	0.05
Three months	17.32 ±12.1	2.78	11.37 ±4.7	1.07	-0.1;12.1	0.06
Six months	12.47 ±7.7	1.76	6.31 ±3.8	0.88	2.2;10.2	0.00
Nine months	12.53 ±8.5	1.94	4.68 ±3.0	0.70	3.6;12.1	0.00
Twelve months	11.95 ±7.1	1.63	4.63 ±3.2	0.74	3.6;11	0.00
PI %						
Baseline	65.89 ± 14.7	3.36	48.37 ±16.8	3.86	0.4;17.6	0.04
Three months	31.66 ±5.5	1.26	30.85 ±5.5	1.26	-2.8;4.4	0.7
Six months	30.27 ±6.2	1.43	28.24 ±5.1	1.18	-1.7;5.8	0.3
Nine months	32.08 ±8.9	2.03	26.77 ±5.2	1.19	0.5;10.1	0.03
Twelve months	30.11 ±6.3	1.44	25.61 ±3.9	0.90	1.0;8.0	0.01

3.2.1 Baseline (Pre-SRD) Results

3.2.1.1 Baseline PPD

Figure 3.2 illustrates the mean and confidence intervals of mean pocket depths (mm) for the test and control groups at baseline. The mean pocket depth in the control group (4.63 ± 0.8 mm) was higher than the test group (4.24 ± 0.6 mm) at baseline; however, this difference was not statistically significant ($p=0.1$) (Table 3.4).

For the percentage of PPDs in each category, (1-3 mm; 4-6 mm; 7-9 mm; 10+ mm,) no statistically significant differences were found between the control and test groups at baseline ($p>0.05$) (Figure 3.3).

Figure 3. 2 Mean PPD (mm) with confidence intervals for control and test groups at baseline (pre-SRD)

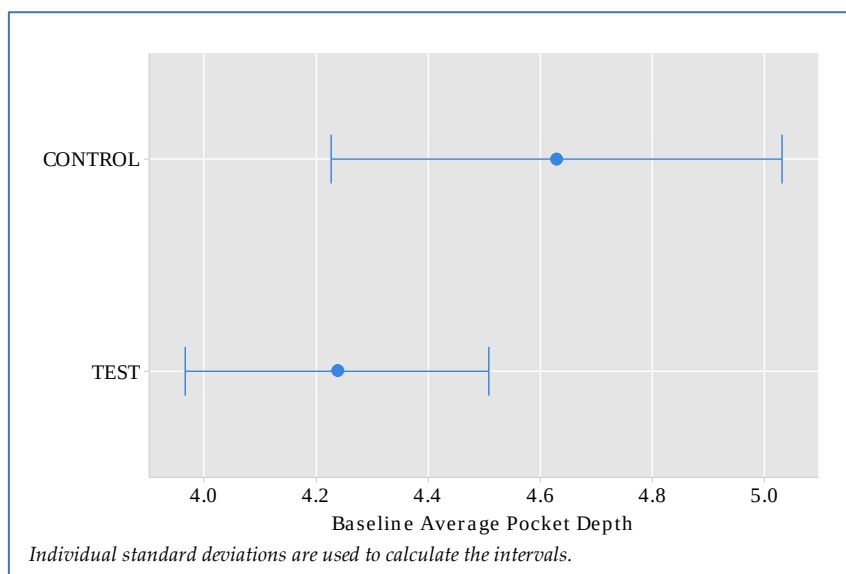
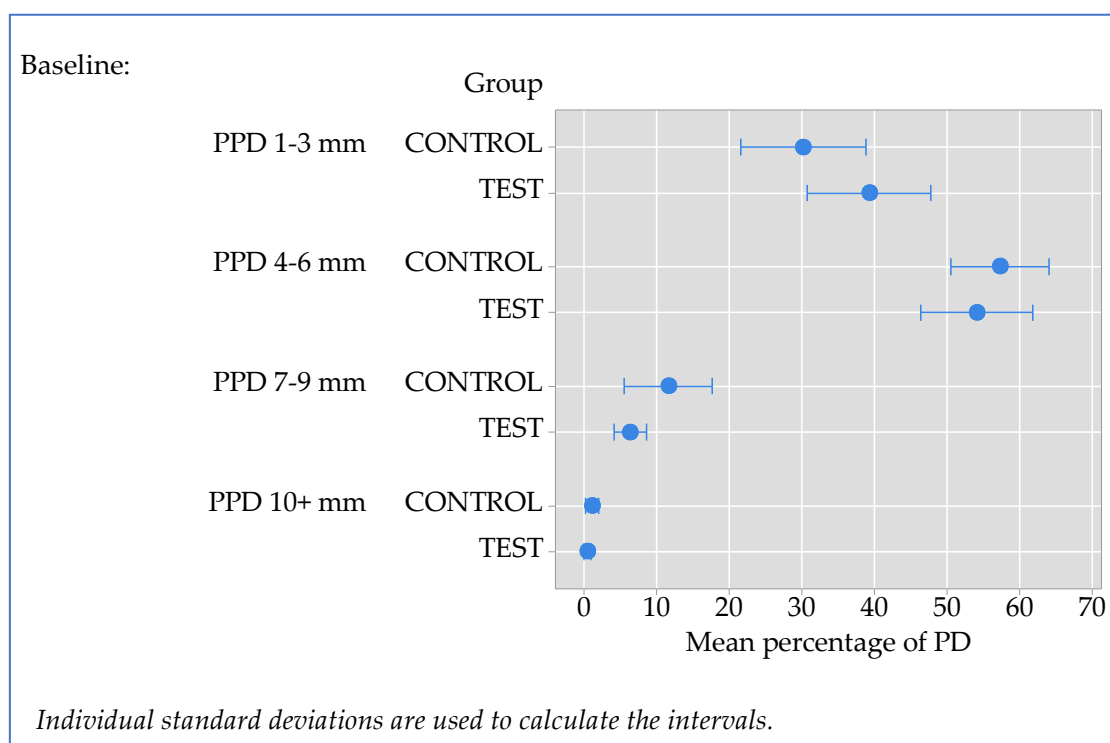


Figure 3. 3 Mean percentage and confidence intervals for PPD 1-3 mm, 4-6 mm, 7-9 mm and 10+ mm for control and test groups at baseline (pre-SRD)



3.2.1.2 Baseline PAL

The mean PAL was higher in the test group (5.13 ± 0.6 mm) as compared to the control group (5.0 ± 0.8 mm) pre-SRD however, this difference was not statistically significant ($p=0.9$) (Figure 3.4) (Table 3.4).

For PAL 1-3 mm, 4-6 mm, 7-9 mm and 10+ mm, there were no statistically significant differences between the groups for the percentage of PAL in each category ($p>0.05$). Figure 3.5 describes mean percentage and confidence intervals for each category of PAL at baseline.

Figure 3. 4 Mean PAL (mm) with confidence intervals for test and control groups at baseline (pre-SRD)

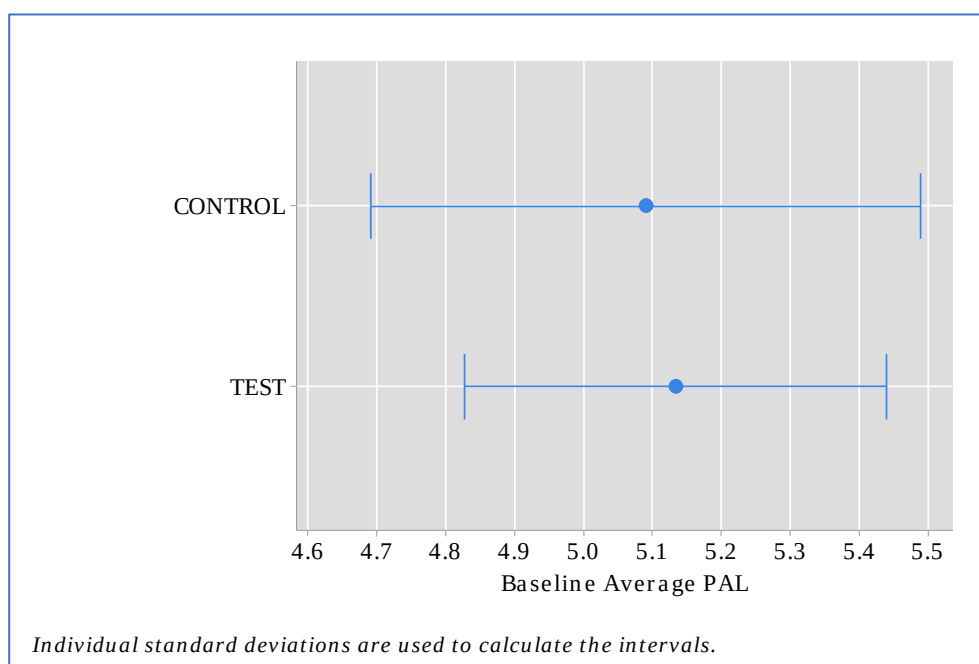
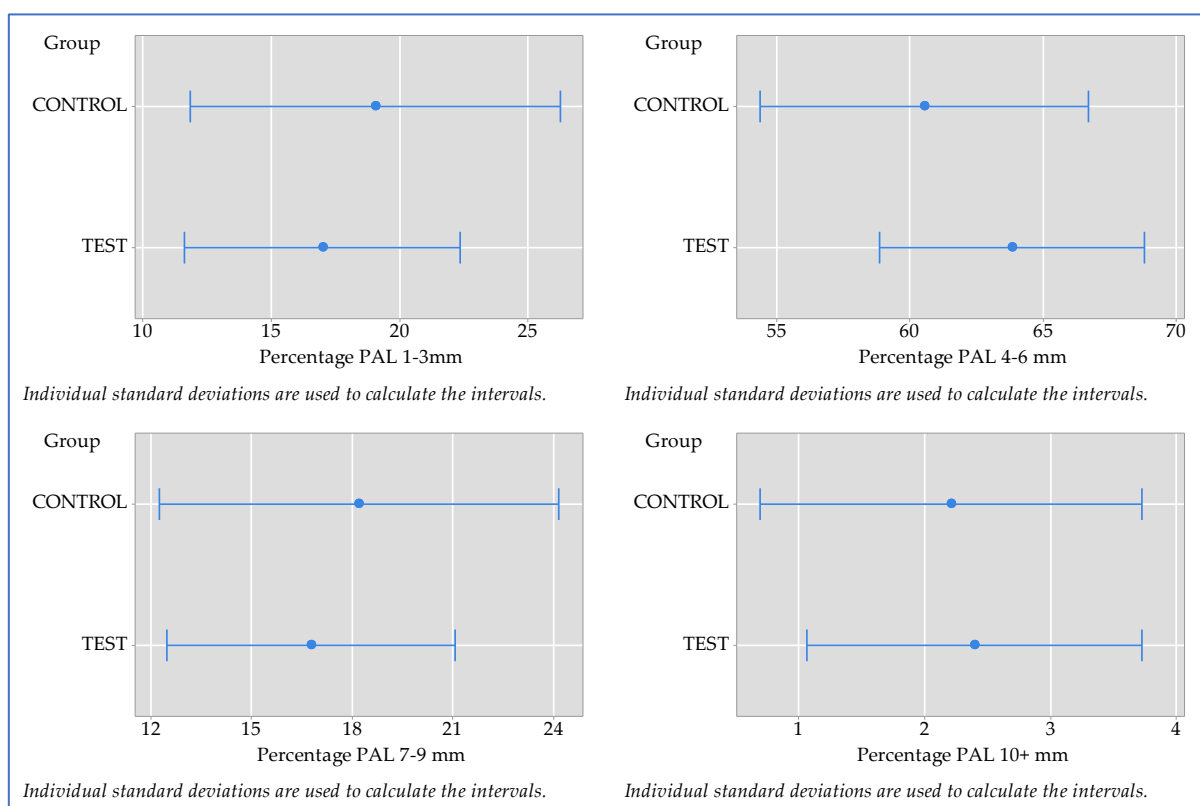


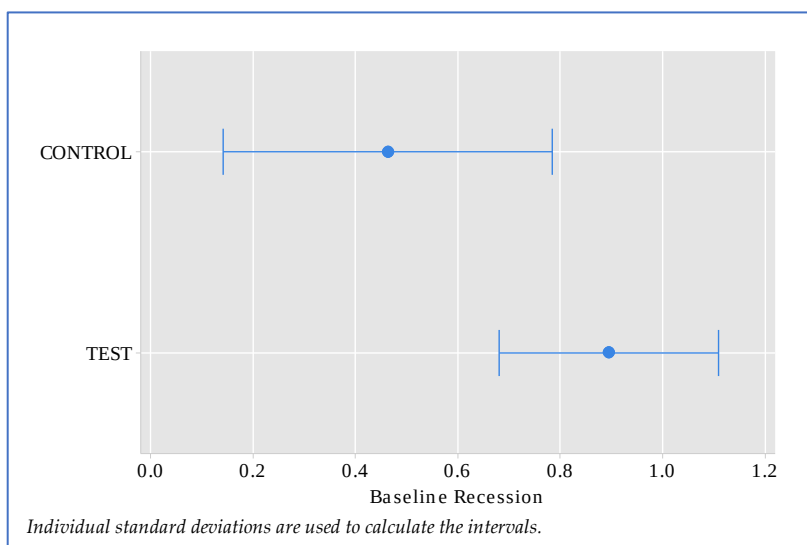
Figure 3. 5 Mean percentage with confidence intervals for PAL1-3 mm, 4-6 mm, 7-9 mm and 10+ mm for control and test groups at baseline (pre-SRD)



3.2.1.3 Baseline Gingival Recession

At baseline, the test group had a statistically significant higher mean recession (0.90 ± 0.4 mm) as compared to the control group (0.50 ± 0.7 mm) ($p=0.03$) (Table 3.4). Figure 3.6 demonstrates mean (mm) and confidence intervals for recession between groups pre- SRD.

Figure 3. 6 Mean recession (mm) and confidence intervals for control and test groups at baseline (pre-SRD)



3.2.1.4 Baseline BOP

BOP was found be higher in the control group ($60.16 \pm 19.6\%$) than the test group ($48.37 \pm 16.8\%$) at baseline. The p-value approached but did not reach statistical significance ($p=0.05$) (Table 3.5).

3.2.1.5 Baseline PI

At baseline, the control group had a statistically significant higher mean percentage PI ($65.89 \pm 14.6\%$) as compared to the test group ($56.86 \pm 11.3\%$) ($p=0.04$) (Table 3.5).

3.2.2 Three Months (Post-SRD) Results

Table 3.4 displays the clinical parameters at three months (post -SRD) for the control and test groups. The mean, standard deviations, standard error of means, confidence intervals, p-values are included. All clinical parameters recorded at baseline were

repeated at three months. Following SRD with or without the perioscope there were significant decreases in all clinical variables ($p<0.05$) at three months.

3.2.2.1 Three months PPD

The mean PPD in the test group (2.89 ± 0.3 mm) was lower than the control group (3.05 ± 0.5 mm) post-SRD. However, this difference in mean PPDs between the groups was not statistically significant ($p=0.2$). The test group had better outcomes at three months than the control group when examining the percentages of PPDs in their categories. The mean percentage of PPDs 1-3 mm at three months was higher in the test group ($82.88\pm7.5\%$) as compared to the control group ($79.28\pm14.8\%$). The mean percentage of PPD (4-6 mm) was lower in the test group as compared to the control group (Table 3.4). The mean percentage of PPDs 10 mm+ was recorded at 0% in the test group post-SRD, with the control group having a few remaining PPDs 10 mm+ ($0.23\pm0.8\%$). However, these differences were not statistically significant ($p>0.05$). For percentage of PPDs 7-9 mm, a statistically significant difference was found between the test and control group ($p=0.04$) with the test group recording a lower mean percentage ($0.7\pm1.2\%$) as compared to the control group ($2.25\pm2.9\%$).

Figures 3.7 and 3.8 compare the mean and confidence intervals at baseline and three months for test and control groups for average PPD (mm) and percentage of sites with PPDs 1-3 mm; 4-6 mm; 7-9 mm; 10+ mm.

Figure 3. 7 Mean and confidence intervals for mean PPD (mm) at baseline (pre-SRD) and three months (post-SRD) for control and test groups

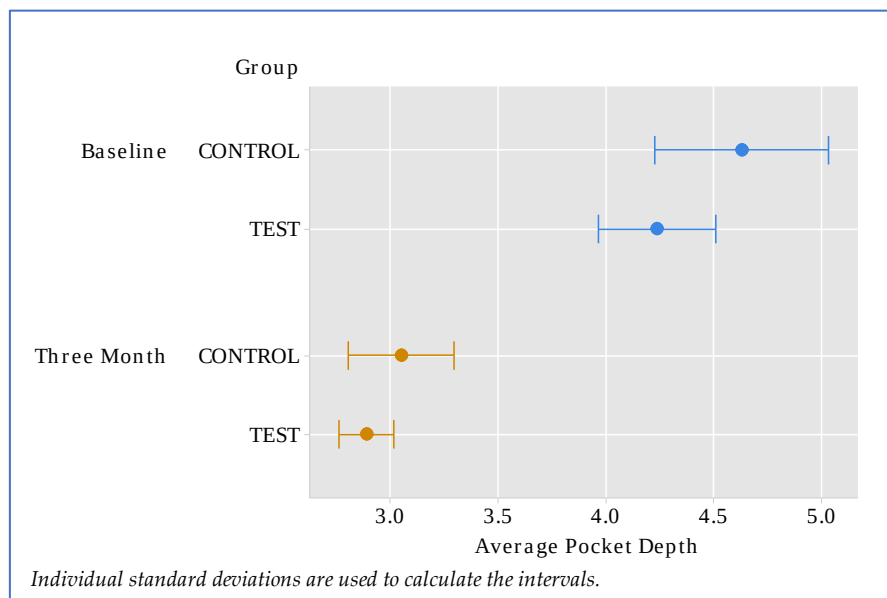
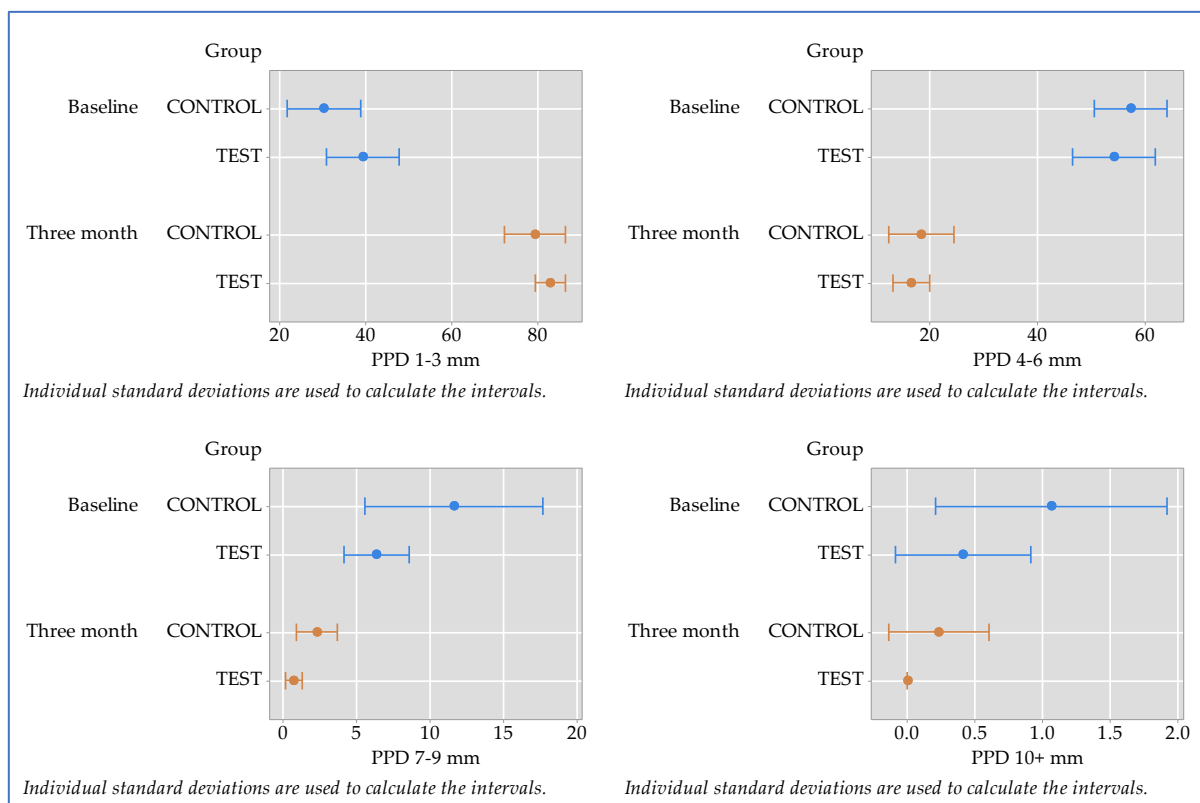


Figure 3. 8 Mean percentage and confidence intervals for PPD 1-3 mm, 4-6 mm, 7-9 mm and 10+ mm at baseline (pre-SRD) and three months (post-SRD) for control and test groups



3.2.2.2 Three months PAL

Figure 3.9 displays the mean and confidence intervals for mean PAL (mm) at baseline and three months for test and control groups.

Mean PAL decreased in both groups at three months; however, the differences between groups did not reach significance ($p=0.9$). There were no statistically significant differences between mean percentage of PAL 1-3 mm; 4-6 mm; 7-9 mm and 10 mm+ between groups at three months ($p>0.05$) (Figure 3.10) (Table 3.4). However, for PAL 10+ mm, the test group recorded a lower PAL in this category than the control group. The p -value bordered on but did not reach significance ($p=0.08$).

Figure 3. 9 Mean and Confidence intervals for average PAL (mm) at baseline (pre-SRD) and three months (post-SRD) for control and test groups

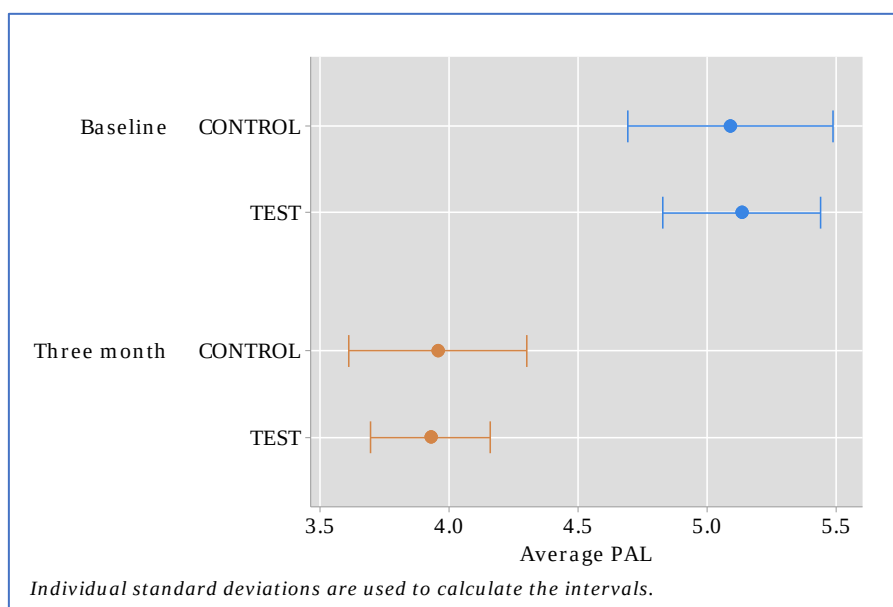
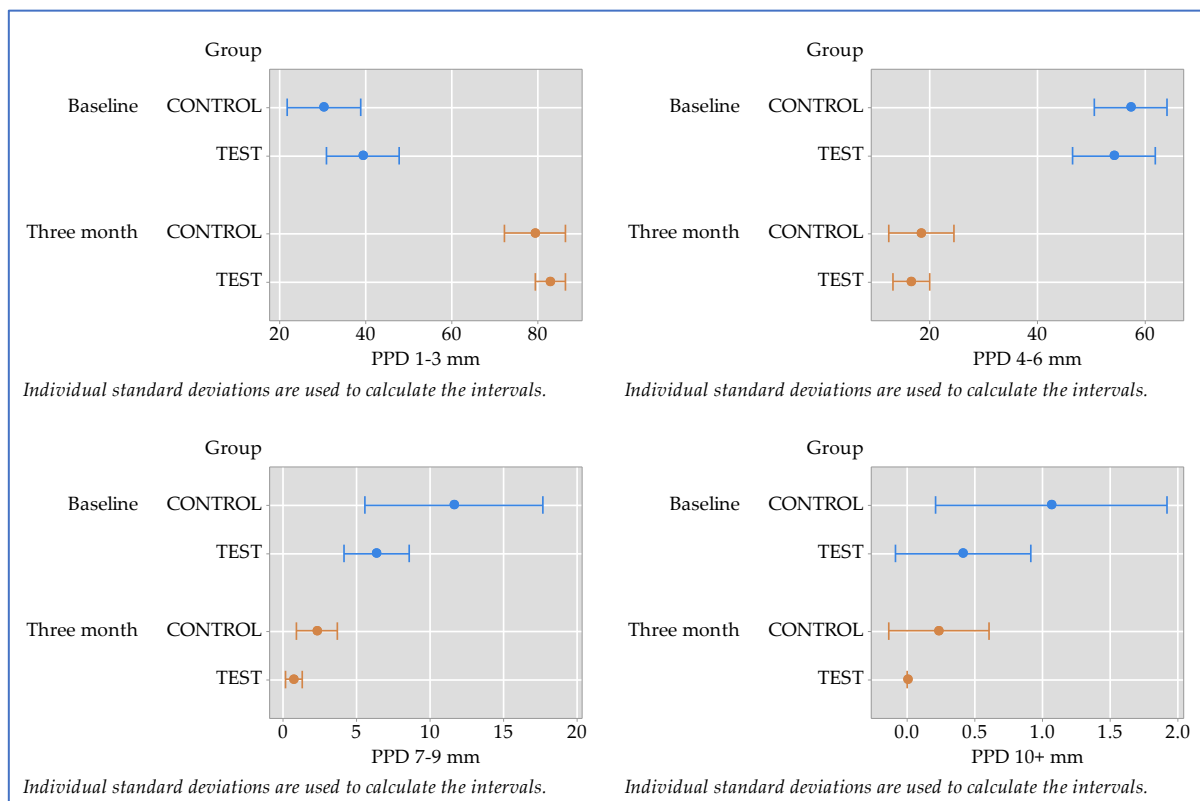


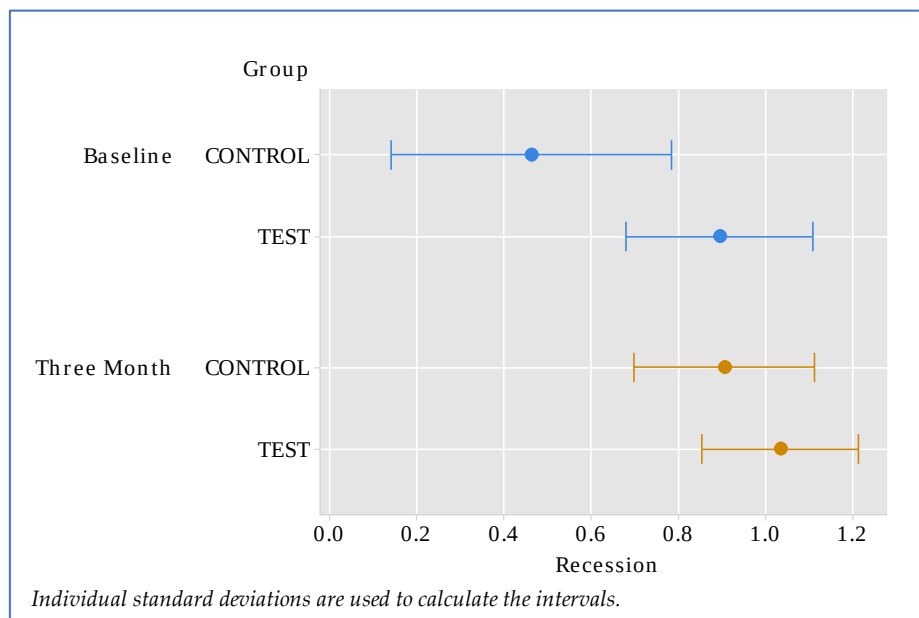
Figure 3. 10 Mean percentage and confidence intervals for PAL 1-3 mm, 4-6 mm, 7-9 mm and 10+ mm at baseline (pre-SRD) and three months (post-SRD) for control and test groups



3.2.2.3 Three months Gingival Recession

From Figure 3.11 it is evident that the mean recession (mm) in the control group increased from 0.50 ± 0.7 mm at baseline to 0.91 ± 0.4 mm at three months, a 0.41 mm increase. In the test group, the increase in mean recession was lower, 0.9 ± 0.4 mm to only 1.0 ± 0.4 mm, a 0.1 mm increase. However, this difference in gingival recession between the test and control groups did not reach significance ($p=0.3$) (Table 3.4).

Figure 3. 11 Mean and confidence intervals for mean recession (mm) at baseline (pre-SRD) and three months (post-SRD) for control and test groups



3.2.2.4 Supportive Periodontal Maintenance Appointments

All thirty-eight participants attended every three months for supportive periodontal maintenance for the 12-month period of the study.

3.2.2.5 Three Months BOP

At three months, mean BOP decreased in both groups ($p < 0.05$). When comparing the difference between mean percentage of BOP in the control group and test group, the mean% BOP in the test group ($11.37 \pm 4.7\%$) was lower than the control group ($17.32 \pm 12.1\%$) and approached but did not reach statistical significance. ($p = 0.06$) (Table 3.5)

3.2.2.6 Six, Nine and Twelve-Month BOP

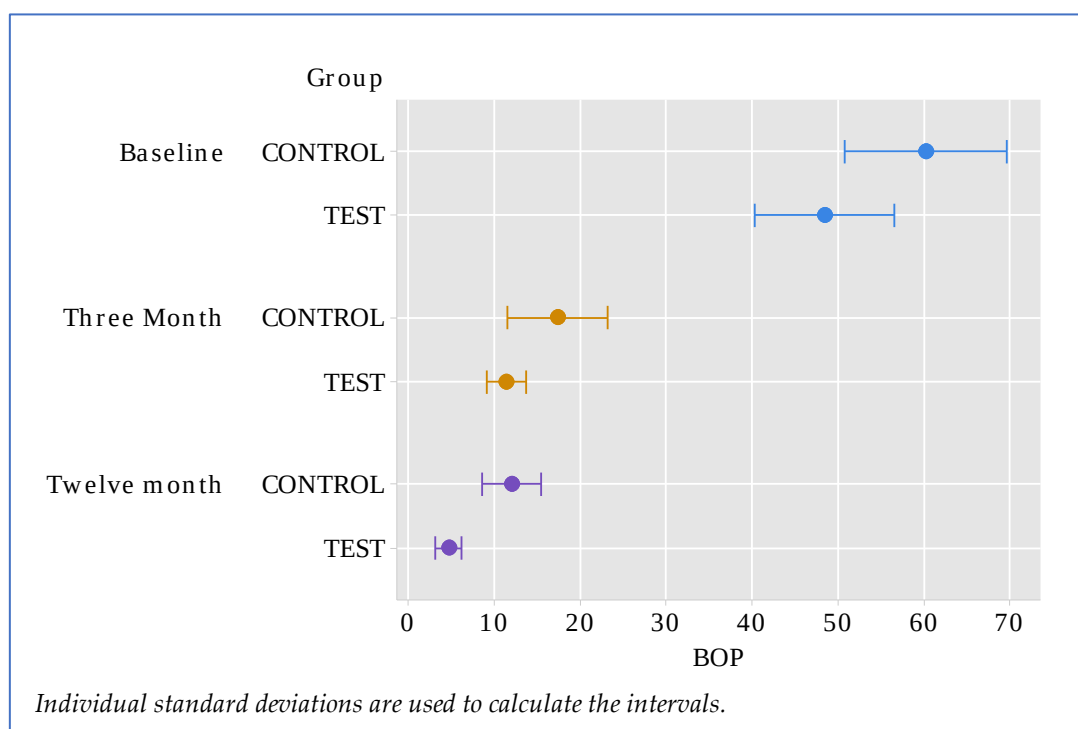
The percentage of sites with BOP were recorded at each SPT appointment. Change in mean percentage of BOP at six, nine and twelve months will be described in this section.

Table 3.5 illustrates the mean percentage of BOP at baseline, three, six, nine and twelve months. At six, and nine months, mean BOP (%) was significantly lower in the test group as compared to the control group ($p=0.00$).

Mean BOP% decreased even further at twelve months in the test group ($4.63\pm3.2\%$) as compared to the control group ($11.95\pm7.1\%$). This difference in mean percentage was statistically significant ($p=0.00$) (Table 3.4).

Figure 3.12 demonstrates the mean percentage and confidence intervals for BOP at baseline, three and twelve months for test and control groups. The figure illustrates the decrease in percentage of sites for BOP in both groups. The mean percentage of BOP decreased significantly more in the test group as compared to the control group at each time interval ($p<0.05$).

Figure 3. 12 Mean percentage and confidence intervals for BOP at baseline (pre-SRD); three (post-SRD) and twelve months for control and test groups



3.2.2.7 Three Months PI

Mean PI (%) decreased in both groups; from $65.89 \pm 14.6\%$ at baseline to $31.66 \pm 5.5\%$ in the control group and in the test group from $56.86 \pm 11.3\%$ at baseline to $30.85 \pm 5.5\%$. This difference between mean PI% between groups did not reach significance ($p=0.6$) post-SRD (Table 3.5).

3.2.2.8 Six, Nine and Twelve-Month PI

The percentage of sites with plaque were recorded at each SPT visit (Table 3.5). OHI were provided at all SPT appointments. At baseline, the control group had a statistically significant higher mean PI% ($65.89 \pm 14.6\%$) as compared to the test group ($56.86 \pm 11.3\%$) ($p=0.04$).

At three months, the mean PI% decreased in both groups; to $31.66 \pm 5.5\%$ in the control group to $30.85 \pm 5.5\%$ in the test group. However, no statistical significance between mean PI% was found between groups. ($p=0.6$).

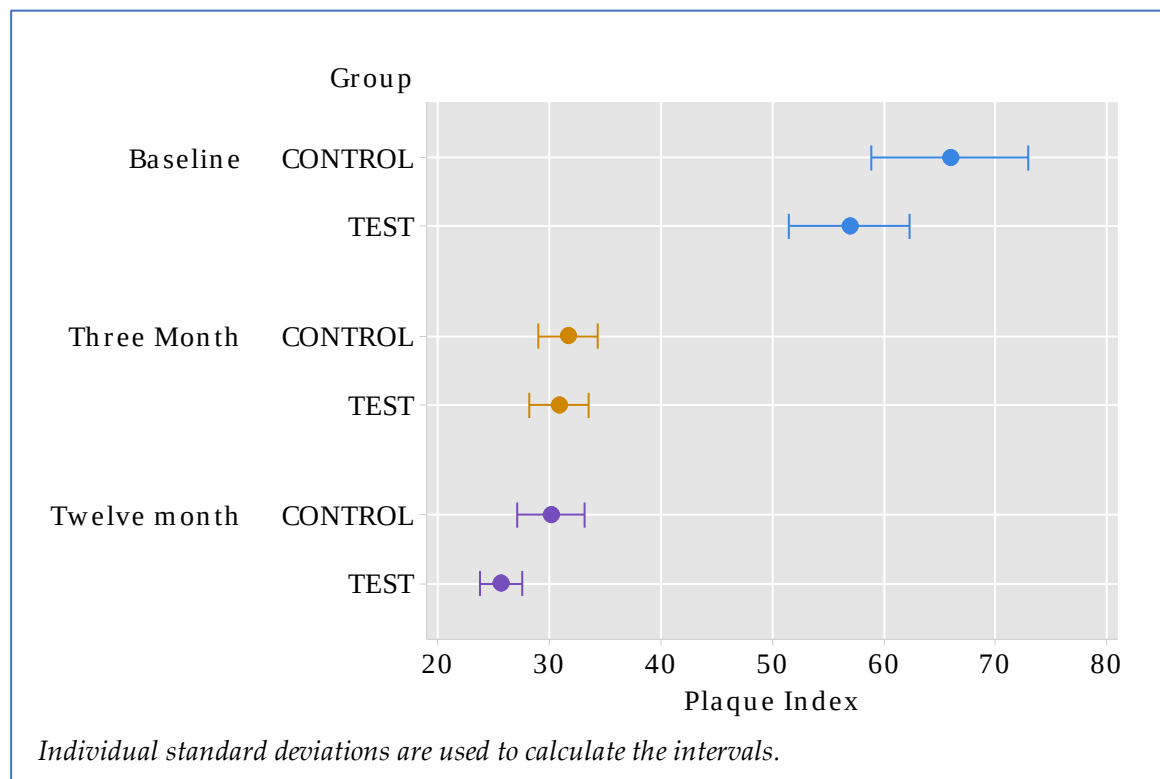
The mean PI% at six months, was found to be lower in the test group ($28.24 \pm 5.1\%$) as compared to the control group ($30.27 \pm 6.2\%$), however this difference in mean PI% was not statistically significant. ($p=0.3$).

At nine months, there was a further decrease in mean % of PI in the test group ($26.77 \pm 5.2\%$) as compared to the control group ($32.08 \pm 8.9\%$). This difference in mean PI% was statistically significant between the test and control groups ($p=0.03$).

This was the same at twelve months, where the test group recorded a lower mean PI% ($25.61 \pm 3.9\%$) as compared to the control group ($30.10 \pm 6.3\%$) and this difference in mean PI% was statistically significant ($p=0.01$) (Table 3.5).

Figure 3.13 illustrates the mean% PI and confidence intervals at baseline (Pre-SRD), three (Post-SRD) and twelve months for test and control groups.

Figure 3. 13 Mean percentage and confidence intervals for PI at baseline (pre-SRD); three (post-SRD) and twelve months for control and test groups



3.2.3 Twelve Month Results

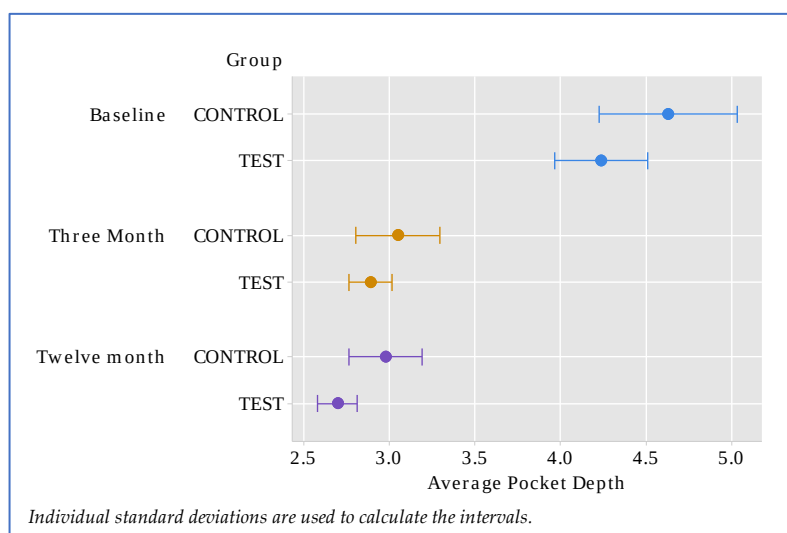
All clinical parameters recorded at baseline and three months were recorded at the twelve-month appointment. There were no exclusions and all thirty-eight participants were included in the twelve-month statistical analysis. Table 3.4 demonstrates the clinical findings at twelve months with the mean, standard deviation, standard error of mean, confidence intervals and p-values illustrated for each group.

3.2.3.1 Twelve Months PPD

Figure 3.14 illustrates the mean PPD (mm) and confidence intervals at baseline (pre-SRD), three (post-SRD) and twelve months for test and control groups. At twelve

months the mean PPD in the test group was 2.70 ± 0.05 mm as compared to the control group 2.97 ± 0.4 mm. This difference in mean PPD between the groups was statistically significant ($p=0.02$) (Table 3.4).

Figure 3. 14 Mean and confidence intervals for mean PPD (mm) at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



At twelve months further improvements in percentage of PPDs in their categories were recorded. Figure 3.15 illustrates the mean % and confidence intervals for the categories of PPD at baseline (pre-SRD), three months (post-SRD) and 12 months for both groups.

For PPDs 1-3 mm, the mean % increased from three months in both groups with the mean % being higher in the test group ($86.66 \pm 6.3\%$) as compared to the control group ($81.59 \pm 13.0\%$). This difference was not statistically significant ($p=0.1$). It was found that at twelve months, that SRD with the use of the perioscope resulted in a significantly higher mean percentage of PPDs 1-4 mm ($95.62 \pm 4.6\%$) as compared to the control group ($89.87 \pm 7.9\%$) ($p<0.05$). (Figure 3.16). Lower mean % PPDs 4-6 mm were reported in the test group ($12.84 \pm 5.9\%$) as compared to the control group ($16.32 \pm 11.4\%$) at twelve months. The test group had no remaining PPDs 10 mm and above. However, these differences were not statistically significant. For PPDs 7-9 mm,

the mean % was significantly lower in the test group ($0.5 \pm 1.0\%$) than the control group ($1.84 \pm 2.3\%$) ($p=0.03$).

Figure 3. 15 Mean percentage and confidence intervals for PPD 1-3 mm; 4-6 mm; 7-9 mm and 10+ mm at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups

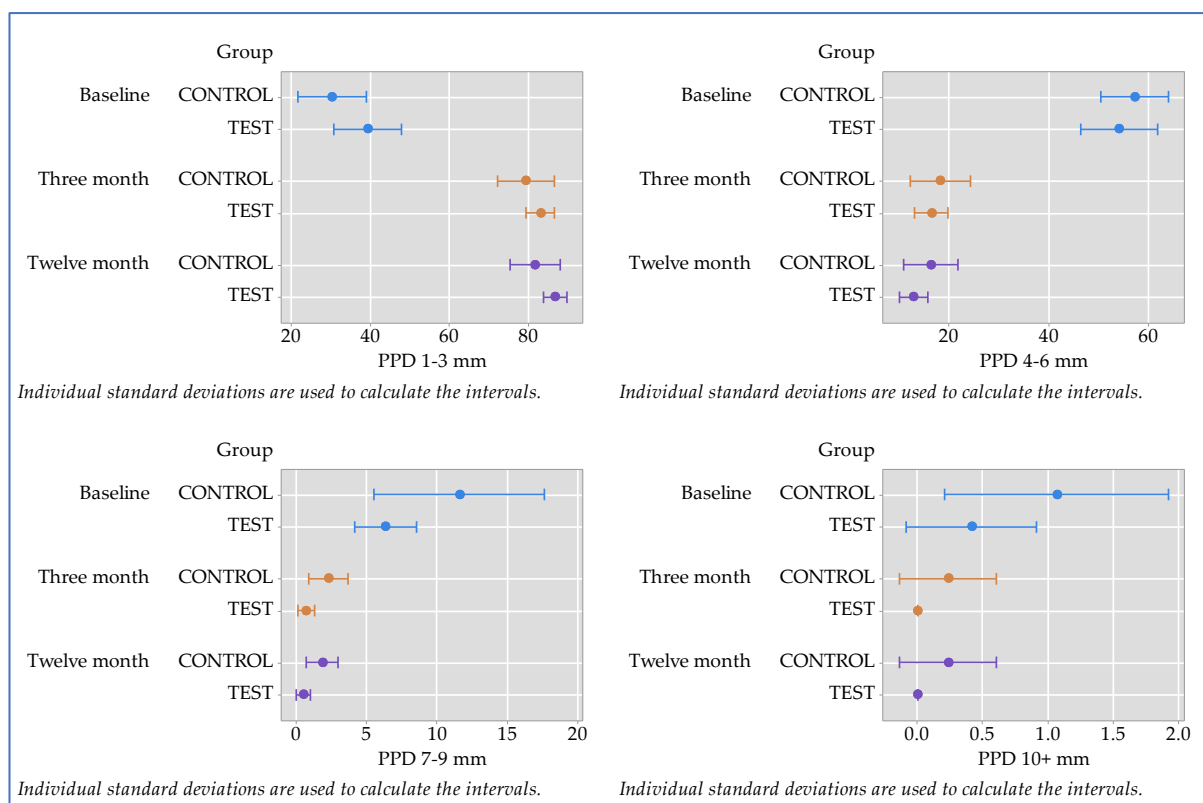
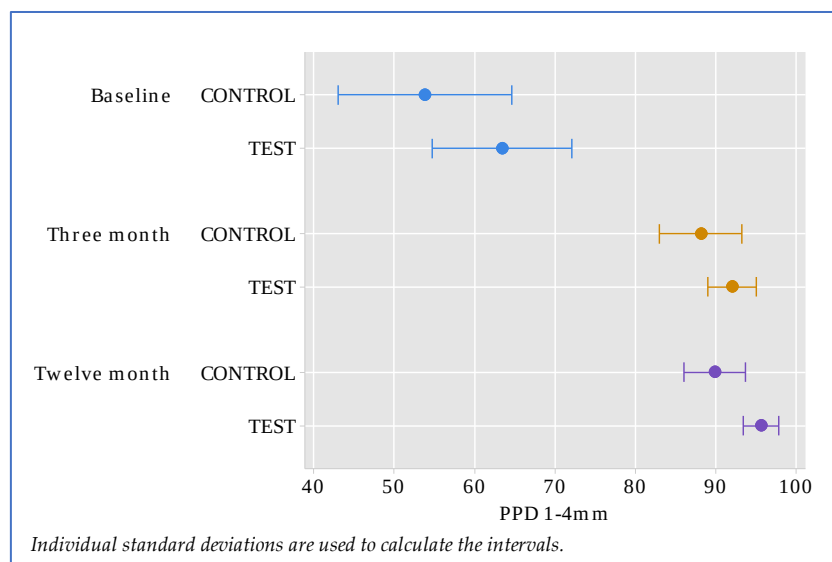


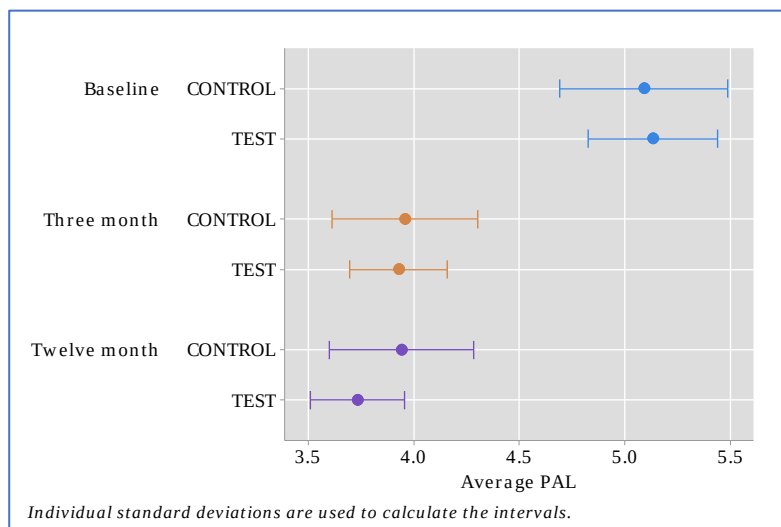
Figure 3. 16 Mean percentage and confidence intervals for PPD's 1-4 mm at baseline (pre-SRD), three months (post-SRD) and twelve months for control and test groups



3.2.3.2 Twelve Months PAL

At twelve months, there were improvements in average PAL and categories in both groups with both groups showing some attachment gain. However, there were no statistically significant differences between the test (3.73 ± 0.5 mm) and control groups (3.94 ± 0.7 mm) for average PAL. (Table 3.4) (Figure 3.17)

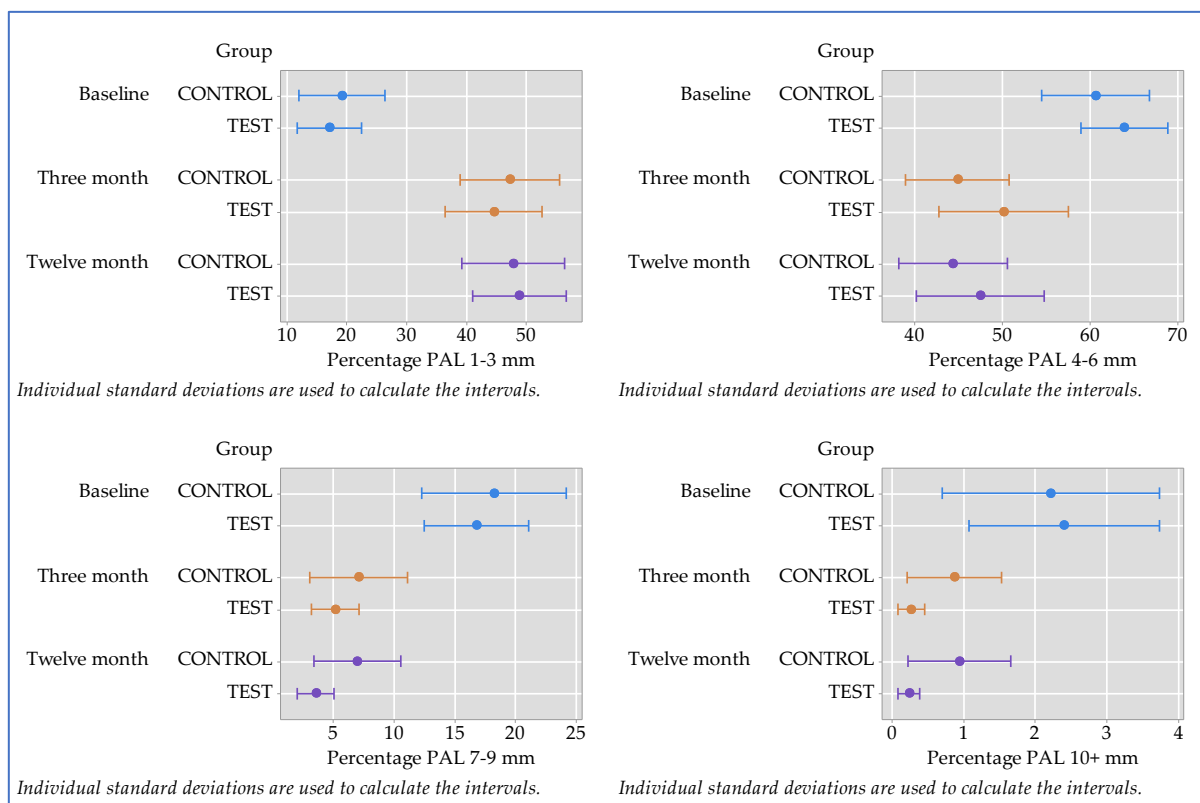
Figure 3. 17 Mean and confidence intervals for mean PAL (mm) at baseline (pre-SRD), three months (post-SRD) and twelve for control and test groups



Mean % of PAL 1-3 mm in the test group was marginally higher (1.04%) but not significantly higher in the test group ($48.81 \pm 16.3\%$) as compared to the control group ($47.77 \pm 17.8\%$). Mean percentage of PAL 4-6 mm was also found be higher in the test group than the control group, however this difference was not statistically significant.

For PAL 7-9 mm and 10 mm+, the mean percentage was lower in the test group as compared to the control group and this difference bordered on significance ($p=0.07$). Figure 3.18 demonstrates the mean and confidence intervals for mean percentage of PAL 1-3 mm, 4-6 mm, 7-9 mm and 10+ mm.

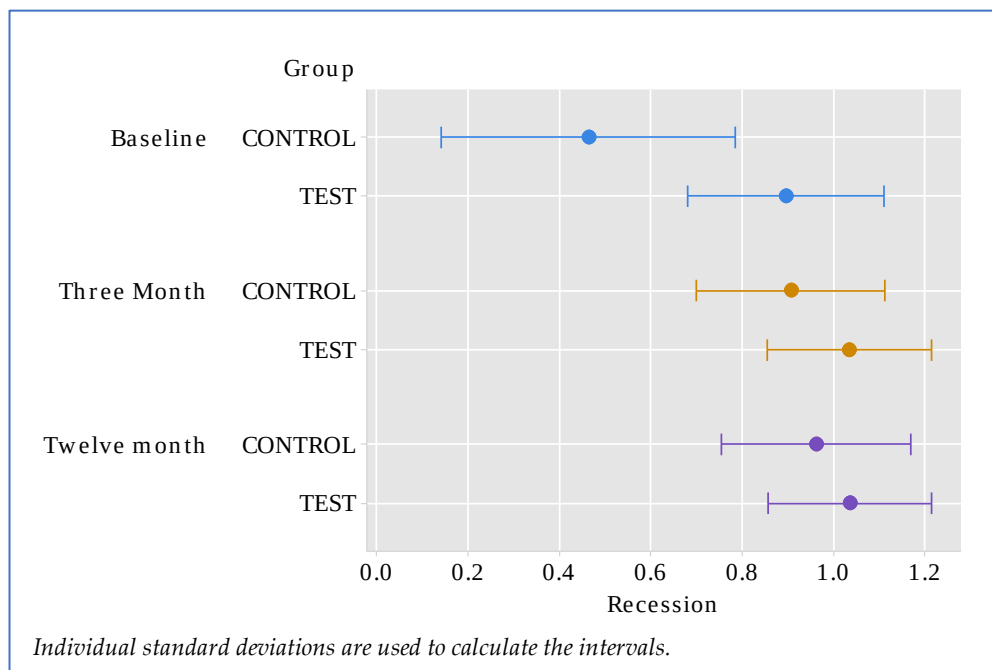
Figure 3. 18 Mean percentage and confidence intervals for PAL 1-3 mm, 4-7 mm, 7-9 mm, 10+ mm at baseline (pre-SRD), three months (post-SRD) and twelve months for control and test groups



3.2.3.3 Twelve Month Gingival Recession

There were no significant differences in mean recession between control (0.96 ± 0.4 mm) and test groups (1.03 ± 0.4 mm) at twelve months ($p=0.5$). Figure 3.19 illustrates the mean and confidence intervals for the average recession at baseline, three and twelve months. When analysing the diagram, negligible differences can be seen when comparing mean recession (mm) from three months to twelve months for both groups.

Figure 3. 19 Mean and confidence intervals for average recession (mm) at baseline (pre-SRD), three months (post-SRD) and twelve months for control and test groups



When analysing the change in recession from baseline (pre-SRD) to twelve months, the change in mean recession in the test group (-0.14 ± 0.2 mm) was much lower than the control group (-0.50 ± 0.6 mm) and this difference was statistically and potentially clinically significant ($p=0.01$). This infers that SRD with the periscope had less recession from baseline to twelve months as compared to the control group. (Table 3.6)

Table 3. 6 Summary of statistics for change in mean recession (mm) from baseline to twelve months

	Control (n=19) SRD - only		Test (n=19) SRD- with periscope		Independent sample t-test summary statistics	
	Mean, standard deviation	Standard error of mean	Mean, standard deviation	Standard error of mean	95% CI	p-value
Change in mean recession from baseline to twelve months						
	-0.50 ±0.6	0.12	-0.13 ±0.2	0.04	-0.6; -0.1	0.01

3.3 Summary of Clinical Findings and Discussion

The success of non-surgical periodontal therapy are measured by clinical outcomes including reduction of PPDs, maintenance or improvement in PALs and reduction of inflammation. However, patient's compliance with home care procedures is also an essential element in the success of periodontal treatment. Over the years there has been many studies conducted using non-surgical periodontal therapy with many reporting positive outcomes following use of this treatment modality (Charalampakis *et al.*, 2013; Cugini *et al.*, 2000; Haffajee *et al.*, 1997b; Hill *et al.*, 1981; Isidor *et al.*, 1986; Loos *et al.*, 1989; Preshaw *et al.*, 1999). Controlled trials comparing the use of the perioscope with conventional SRD in reducing clinical parameters are limited. The current study's aims were to compare these two types of non-surgical periodontal therapy methods. The perioscope allows the clinician to identify and view subgingival deposits and it was hypothesized that this adjunct for calculus detection and removal could result in more positive clinical outcomes as compared to conventional non-surgical therapy. We have compared data for the current study to clinical trials utilizing non-surgical debridement and also to the few studies utilizing the perioscope. The current study examined the effects of both non-surgical treatment methods on clinical outcomes including PPD, PAL, recession, BOP and PI and compared the use of SRD-with perioscope with SRD-only in improving clinical outcomes.

The clinical analysis included 38 patients. The mean age of study population was 52.50 ± 11.05 years with 24 females and 14 males. There were no significant differences between groups with regards to age, gender, medical history or disease severity. The majority of patients were non-smokers (84.2%); and even though the test group had twice as many smokers as the control group, these differences were not significant (5.3% in the control group; 10.5% in the test group). With such a small percentage of smokers in the study population, the analysis did not include smoking as a confounding factor as analysis including smokers did not impact greatly on the outcomes of this study.

Although the same selection criteria were followed for all participants, there were some clinical differences between the two groups at baseline which is relevant to some of the clinical outcomes. The SRD-only group had a higher mean PPD, more gingival

inflammation and higher plaque levels than the SRD with perioscope group. However, these differences were not statistically significant. In addition, the mean recession in the test group (0.90 ± 0.4 mm) was found to be significantly higher than the control group (0.46 ± 0.7 mm) at baseline ($p=0.03$).

The reduction of PPDs occurs as a result of gain in clinical attachment levels, gingival recession (Hughes *et al.*, 1978; Proye *et al.*, 1982) as well as reduction in inflammation of the gingival tissues (Robinson *et al.*, 1979). For this clinical trial, both treatment modalities resulted in reduction of all clinical variables post-therapy. Further reductions in both groups were also observed at the twelve-month re-evaluation appointment. However, when comparing the test and control groups, differences in clinical outcomes were observed at both time points.

3.3.1 Effect of SRD-only and SRD-with perioscope on Probing Pocket Depths

The greatest change in probing depth reduction and PALs usually occurs within 1-3 months following SRD (Badersten *et al.*, 1981; Badersten *et al.*, 1984a; Cugini *et al.*, 2000; Kaldahl *et al.*, 1988; Morrison *et al.*, 1980; Preshaw *et al.*, 1999). At the start of the clinical trial, even though there were no significant differences between mean PPD between groups; the control group had a higher mean PPD (4.63 ± 0.8 mm) as compared to the test group (4.24 ± 0.6 mm). The mean PPD decreased in both groups post-SRD with the test group having a lower mean PPD (2.89 ± 0.3 mm) as compared to the control group (3.05 ± 0.5 mm). However, these differences were not statistically significant. When examining the reduction of the mean PPD from baseline (pre-SRD) to three months (post-SRD), a higher mean reduction in PPD was observed in the control group (1.58 mm) as compared to the test group (1.35 mm). Studies have demonstrated that factors such as initial PPDs, deeper PPDs, patient's oral hygiene, tooth anatomy, etc. can all influence the outcome of non-surgical periodontal therapy (Cobb, 1996{Badersten, 1984 #491}). The greater reduction in the mean PPDs in the control group possibly reflects the deeper pockets in this group at baseline.

When comparing the mean reduction in PPD recorded post-SRD for this clinical trial to other studies using conventional non-surgical debridement, the following

associations can be made. Hämmerle *et al.*, (1991) had a lower mean probing depth reduction of 0.66 mm from 3.96 ± 1.39 mm at baseline to 3.30 ± 1.16 mm after therapy as compared to the current trial (Hämmerle *et al.*, 1991). Badersten, *et al.*, (1981) reported a 1.3 mm to 1.7 mm reduction in mean PPDs four to five months post-therapy (Badersten *et al.*, 1981). The mean probing depth reduction was 1.25 mm in the Sherman *et al.*, (1990) study in pockets depths ranging from 3.5 mm to 6 mm (Sherman *et al.*, 1990). In non-smokers a 1.7 ± 1.4 mm reduction in mean PPDs was observed in the clinical trial by Darby *et al.*, (2005) (Darby *et al.*, 2005). The mean reduction of PPDs in this study is consistent with previously published reports of 1.2 mm to 2.9 mm (Becker *et al.*, 2001; Hämmerle *et al.*, 1991; Morrison *et al.*, 1980; Pihlstrom *et al.*, 1983; Ramfjord *et al.*, 1987).

There are a limited number of studies available for comparison between SRD-only and SRD with the use of the perioscope. The pilot study by Blue *et al.*, (2013) compared the outcome of SRD with or without the perioscope at 6-8 weeks and three months post-therapy. Thirty patients were recruited for this randomised split mouth study design with 26 patients completing the treatment. It was found that mean PPD reduced by 1.43 mm (from 5.29 ± 0.4 mm to 3.86 ± 0.6 mm) by visit 1 and by another 0.31 mm (3.5 ± 0.8 mm) at visit 2 in the “perioscope sites”. In non-perioscope sites, mean PPD reduced by 1.48 mm (from 5.39 ± 0.5 mm to 3.91 ± 1.2 mm) at visit 1 and by 0.08 mm (3.83 ± 1.2 mm) at visit 2. Lower mean PPDs were observed in the perioscope sites as compared to the non-perioscope sites. However no significant differences were found between the two treatment modalities used (Blue *et al.*, 2013). Similarly, our study also found that the SRD-with perioscope group had a lower mean PPD as compared to the SRD-only group with no significant differences observed between the two groups post-SRD. When comparing the mean reduction in PPDs post therapy for the current study to Blue *et al.*, (2013), the mean reduction in the SRD-only group was similar (1.58 mm) when compared to their study (1.56 mm). However, the mean reduction in PPDs in the SRD-with perioscope group was 1.35 mm which is lower than reported in the Blue *et al.*, study (1.74 mm). However, the greater reduction in mean PPDs in Blue *et al.*, (2013) could be related to higher mean PPDs (5.29 ± 0.4 mm) before commencement of treatment. Hou (2016) compared the effects of periodontal endoscopy to traditional scaling using RCT, parallel design. At the three month f/up appointment, no significant differences in PPD reduction was observed between the two groups (Hou.Y, 2016).

Avradopoulos *et al.*, (2004) found no significant differences between the SRD alone and SRD-with perioscope group. This clinical trial was a split-mouth design and included six patients. At one-month post-therapy, even though the SRD with adjunctive use of the perioscope had higher percentage of pocket depth reduction than SRD-only, the difference was not statistically significant ($p=0.76$). At three months, this study reported 55% (12 of the 22 sites) of sites with PPD reduction >2 mm in the control group compared to 46% (10 of the 22 sites) of the experimental sites. However, no statistical significance were reported ($p=0.55$). The limitations described for this study was the small study population and short f/up (Avradopoulos *et al.*, 2004). The RCT split design study by Liao *et al.*, (2016) found that the use of perioscope was more effective for anterior teeth with deep PPDs ≥ 6 mm with the test group having a significantly lower PPD (3.2 ± 0.9 mm) as compared to the control group (3.7 ± 0.9 mm) at three months (Liao *et al.*, 2016).

Even though the greatest change in probing depth reduction and gain in clinical attachment levels occurs in the first three months following treatment, healing and maturation of the periodontium may continue over 9-12 months (Badersten *et al.*, 1981; Badersten *et al.*, 1984a; Cugini *et al.*, 2000; Kaldahl *et al.*, 1988; Morrison *et al.*, 1980). Haffajee *et al.*, (1997) reported that improvements in both PPDs and clinical attachment levels continued at later visits following the three month re-evaluation (Haffajee *et al.*, 1997b). This was a similar finding in the Cugini *et al.*, (2000) study where it was reported that the mean probing depths continued to improve during their twelve month study (Cugini *et al.*, 2000). The results of the current study are consistent with previous research and also shows that mean PPDs' continued to improve in both groups and further reductions in mean PPDs were observed in both groups at the twelve-month re-evaluation appointment. The test group recorded a statistically significant lower mean PPD (2.70 ± 0.2 mm) at twelve months as compared to the control group (2.98 ± 0.2 mm). The test group had a further 0.28 mm reduction in mean PPD from three to twelve months, whereas the control group recorded a 0.07 mm reduction. It can be said that healing and maturation of the pockets continued for both groups, with the test group slightly better at twelve months. Further reduction in mean pocket depths following non-surgical debridement was also reported in the Baelum *et al.*, (2016) study. A mean reduction of 0.2 ± 0.2 mm was observed from three to twelve

months in this study (Baelum *et al.*, 2016). Similarly, Cugini *et al.*, (2000) reported a 0.2 mm reduction of mean PPDs from three to twelve months (Cugini *et al.*, 2000). For the current study, the SRD-with perioscope group had a higher mean reduction of PPD (0.28 mm) as compared to these studies.

Studies have shown that improvements occur the most in deepest pockets with moderate pockets having somewhat less improvement (Cobb, 2002; Drisko, 2001; Farman *et al.*, 2008; Heitz-Mayfield *et al.*, 2002; Van der Weijden *et al.*, 2002). To gain a better understanding into the reduction of PPDs between the two treatment groups, PPDs were categorised into PPD 1-3 mm, 4-6 mm, 7-9 mm and 10+ mm and compared between groups at pre-SRD, post-SRD and at twelve months.

When examining the percentage of sites with PPDs 1-3 mm, both treatment modalities resulted in increases in percentages post-SRD. The control group had a 49.14% increase in PPDs 1-3 mm post-SRD, while the test group, recorded a 43.67% increase for PPDs 1-3 mm. At twelve months, further increases in mean percentage of PPDs 1-3 mm were observed in both groups with the control group increasing from 79.28% post-SRD to 81.59% at twelve months (2.31% increase), whilst the test group had a greater increase from 82.88% post-SRD to 86.66% at twelve months (3.78% increase). The difference in mean percentage of PPDs 1-3 mm between groups were not statistically significant post-SRD and at twelve months. The results of the current study for percentage of PPDs 1-3 mm post-SRD is higher than that reported by Mlachkova *et al.*, (2014). Mlachkova included 30 patients with moderate chronic periodontitis, treated with SRD and 0.2% chlorhexidine gluconate rinses twice daily and found that 8-10 weeks post -therapy, PPDs 1-3 mm increased by $35 \pm 2.46\%$ from 1783 sites ($49.45\% \pm 5.20$) at baseline to 3046 sites ($84.47\% \pm 6.43$) post-therapy (a $35.00\% \pm 2.46$ increase ($p < 0.001$)). The authors related this improvement in PPDs 1-3 mm to mechanical debridement (Mlachkova *et al.*, 2014). A systematic review by Heitz-Mayfield *et al.*, (2002) found that SRD is effective in reducing PPDs 1-3 mm with less AL recorded as compared to surgical procedures (Heitz-Mayfield *et al.*, 2002). The current study suggests that both treatment modalities resulted in increases in PPDs 1-3 mm post SRD. Healing of the pockets continued in both groups with increases in percentage of sites with PPDs 1-3 mm observed at twelve months. One can assume that additional healing took place in the SRD-with perioscope group as percentage of

sites with PPDs 1-3 mm was higher as compared to the SRD-only group at twelve months.

Pocket depths 4 mm or less are easier to maintain together with good oral hygiene practices (Waerhaug, 1978). It was found that at twelve months, that SRD-with perioscope resulted in a statistically significant higher mean percentage of sites with PPDs 1-4 mm ($95.62 \pm 4.6\%$) as compared to SRD-only ($89.87 \pm 7.9\%$) ($p < 0.05$) (Figure 3.17). In the test group, less than 5% of sites had PPDs greater than 4 mm and in the control group, 10.13% of sites had PPDs greater than 4 mm.

For moderate pockets 4-6 mm, the control group had a 38.99% reduction as compared to 37.64% in the test group from baseline to three months. Initial pocket depth does affect the outcome post-SRD and in this study the control group had a greater percentage of PPDs 4-6 mm at pre-treatment. However, at twelve months, the test group had a further 3.58% reduction in PPDs 4-6 mm from three months to twelve months as compared to 0.1% reduction in the control group, again suggesting that healing continued to take place in both groups with the test group recording more reductions at twelve months.

Adriaens and Adriaens (2004), in their review of the effect of non-surgical periodontal therapy on hard and soft tissues analysed 28 studies and found that for non-molar defects with initial PPDs 4-6 mm, a mean reduction of PPD of 1.29 mm was achieved and mean gain in PAL was 0.55 mm. The mean reduction in PPDs ranged from 0.3 mm to 2.1 mm. For molar sites the mean PPD reduction varied from 0.00 mm to 1.02 mm and mean changes in PAL varied between a loss of 0.80 to a gain of 0.28 mm (Adriaens *et al.*, 2004).

There are limited studies available to compare the pocket depth reduction that occurs at three and twelve-months post-therapy utilising the perioscope. A larger study by Kwan and Workman (2009) was a non-blinded prospective study utilising the perioscope for non-surgical management of 270 patients with moderate to advanced periodontal disease. Treatment was completed in one visit and systemic antibiotics were provided post-treatment. In their study, it was found that for molars with initial PPDs 5-6 mm, 69% reduced to <4 mm at the twelve months recall appointment ($n=478$ teeth). For premolar teeth with initial PPD 5-6 mm, 78% reduced to <4 mm at twelve

months (n=266 teeth). 92% of anterior teeth with PPDs 5-6 mm reduced to ≤ 4 mm (n=246). This study concluded that endoscopic debridement together with the use of systemic antibiotics is effective way of managing moderate to advanced periodontal disease (Kwan *et al.*, 2009). Our study did not analyse anterior, premolar and molar teeth individually. However, when comparing the reduction of mean percentage of PPDs 4-6 mm, the control group recorded a 39.09% decrease in PPDs 4-6 mm from baseline to twelve months, and the test group had a 41.22% decrease in PPDs 4-6 mm. This is lower than reported in the Kwan and Workman (2009) study, however, one has to consider that a larger study population was examined in their study and systemic antibiotics were used. The study by Stambaugh and Myers (2003) utilised the perioscope to debride 476 pockets measuring 4-6 mm. PPDs assessed 21 months later reported a mean reduction of 2.43 mm in PPDs 4-6 mm (Stambaugh *et al.*, 2003).

For the current study, for deep pockets (PPD 7-9 mm), the SRD-with perioscope resulted in a significantly lower mean percentage post-SRD with only 0.7% of sites with remaining PPDs 7-9 mm as compared to 2.25% in the control group ($p < 0.05$). Further reductions in percentages were seen at twelve months, with the test group only recording 0.5% PPDs 7-9 mm and the control group having 1.85% of sites with PPDs 7-9 mm. The results of the current study suggest that SRD-with perioscope was more effective in reducing PPDs 7-9 mm as compared to SRD-only. The further reduction of PPDs 7-9 mm at twelve months could be associated with the longer healing time in deeper periodontal pockets (Badersten *et al.*, 1984b).

When comparing our results to studies utilizing conventional non-surgical debridement, the following information can be extrapolated. Badersten *et al.*, (1991) reported that significant reduction in deep pockets ≥ 6 mm took place irrespective of operator or method of instrumentation. PPDs ≥ 6 mm reduced from 106 at baseline to only 13 remaining at 13 months (88% reduction) (Badersten *et al.*, 1981). Our study had an 84% reduction for PPDs 7-9 mm in the SRD-only group and a 92% reduction in the SRD-with perioscope group from baseline to twelve months. In molar sites with PPDs greater than 7 mm, the mean reduction in PPD ranged from 0.00 mm (Loos *et al.*, 1989) and 1.52 mm (Claffey *et al.*, 1990) following non-surgical periodontal therapy. Cobb reported a mean reduction between 1.2- 2.9 mm in PPDs more than 7 mm post-SRD (Cobb, 1996).

The statistically significant reduction of PPDs 7-9 mm in the current study can be compared to the studies utilising the perioscope by Kwan and Workman (2009), Liao *et al.*, (2016), and Stambaugh and Myers (2003). The results of Kwan and Workman (2009) showed that 55% of molars with PPDs 7-9 mm, reduced to <5 mm at one year (n=284); 57% of premolars with PPDs 7-9 mm reduced to <5 mm (n=30) and 89% of anterior teeth reduced to <5 mm (n=57) (Harrel *et al.*, 2015; Kwan *et al.*, 2009). Similar findings were reported by Liao *et al.*, (2016) with significant differences between SRD-only and SRD with the use of the perioscope for PPDs ≥ 6 mm. Using a split mouth design, eleven patients were randomly placed into test group (SRD with the perioscope) and control groups (SRD-only). PPDs were found to be significantly lower for anterior teeth with PPD ≥ 6 mm in the test group (3.2 ± 0.9 mm) as compared to the control group (3.7 ± 0.9 mm). No other significant differences were reported with other clinical parameters used. This study concluded that the use of the perioscope was effective especially for medium to long term management of anterior teeth with deep pockets (Liao *et al.*, 2016). The study by Stambaugh and Myers (2003) reported a 5.25 mm reduction in PPD for PPDs greater than 6 mm (Stambaugh *et al.*, 2003).

PPDs >7 mm remained in SRD quadrants (5% at 12 months) (Lindhe *et al.*, 1985) with studies showing that conventional SRD was not as effective in reducing deeper pockets as compared to surgical procedures (Becker *et al.*, 1988; Lindhe, 1982). Badersten *et al.*, (1990) in their study found that residual PPDs >7 mm, showed an improved predictive value for probing attachment loss and reached 52% after 60 months (Badersten *et al.*, 1990). This was further supported in the systematic review by Heitz-Mayfield *et al.*, (2002) where it was concluded that deeper pockets can be difficult to debride using conventional SRD and surgical procedures may result in greater gain in pocket depth reduction and clinical attachment levels (Heitz-Mayfield *et al.*, 2002). The current study supports the effectiveness of the use of the perioscope in PPDs 7-9 mm. Our findings are suggestive that SRD with the use of perioscope is more effective in reducing the deeper pockets 7-9 mm as compared to conventional SRD. One can speculate that the direct visualization of the root surface with the use of the perioscope can result in successful debridement of deeper pockets and may reduce the need for surgical periodontal treatment for these areas.

There were only a few pocket depths ranging from 10+ mm for this clinical trial. Both groups, displayed reductions in mean percentage of sites with PPDs 10 mm+ post-SRD with the control group reducing from 1.07% to 0.23% and the test group (0.41% to 0.00%). No further reductions in PPDs 10+ mm was observed in the control group at twelve months. In the test group, PPDs 10+ mm was completely eliminated with 0% reported at three and twelve months. Badersten *et al.*, (1984) found that in deep pockets up to 12 mm, PPDs reduced by 0.9-1.2 mm at three months. This study also reported that periodontal conditions continued to improve 6-9 months following instrumentation (Badersten *et al.*, 1984b). In the study by Kwan and Workman (2009), 71% of molar sites with PPDs 10-11 mm, reduced to 6-9 mm and 20% reduced to ≤ 5 mm (n=45). In premolars, 38% reduced to ≤ 5 mm (8 teeth) and in anterior teeth, 71% reduced to ≤ 5 mm (n=7). In this study, anterior teeth with deeper pockets responded much better than pre-molar and molar teeth (Harrel *et al.*, 2015; Kwan *et al.*, 2009). Kwan and Workman (2009) had a larger study population as compared to the current study and systemic antibiotics were also utilised. In addition, the only treatment of choice was perioscopy and there was no comparison to conventional SRD.

Even though studies comparing the use of the perioscope with conventional debridement reported greater reduction in pocket depths (Avradopoulos *et al.*, 2004; Blue *et al.*, 2013), the reductions were not found to be statistically significant post-SRD. Only Liao *et al.*, identified benefits of periodontal endoscopy as compared to conventional SRD in anterior teeth with deep pockets (Liao *et al.*, 2016). The present study reports a statistically significant reduction in mean PPD at twelve months in the SRD-with perioscope group and also identifies the benefits of the use of the perioscope in PPDs 7-9 mm.

3.3.2 Effect of SRD-only and SRD-with perioscope on Probing Attachment Level

PALs are influenced by the amount of gingival recession as well as pocket depth. The extent of probing depth reduction and attachment levels gains are typically correlated to the initial measurements (Cobb, 1996). In the current study, even though the control group had a higher mean PPD pre-SRD, the mean PAL was lower in the control group (5.09 ± 0.8 mm) as compared to the test group (5.13 ± 0.6 mm). When examining the mean recession, it was found that the test group had a significantly higher mean recession (0.90 ± 0.4 mm) as compared to the control group (0.46 ± 0.7 mm) which in turn affected the PAL.

At three months (post-SRD), the mean PAL reduced in both groups. This is similar to the study by Cugini *et al.*, (2000) which also showed significant reductions in PAL three months post-SRD (Cugini *et al.*, 2000). The mean PAL reduced by 1.13 mm in the control group; whereas in the test group it reduced by 1.2 mm. The mean PAL for the current study is higher than reported by Haffajee *et al.*, (1997) who reported a mean gain of 0.11 ± 0.23 mm with attachment levels ranging from 0.53 mm loss to a gain of 0.64 mm at the three months re-evaluation following conventional non-surgical therapy (Haffajee *et al.*, 1997b).

Further gains in attachment were observed in both groups at twelve months. The gain in attachment from baseline to twelve months in the control group was 1.15 mm and in the test group, 1.4 mm. When analysing attachment gain from three to twelve months, gain in attachment in the control group was only 0.02 mm; whereas in the test group, attachment gain increased by 0.2 mm, suggesting more attachment gain in the SRD-with perioscope group as compared to the SRD-only group at twelve months. Baelum and Lopez (2016) observed a gain in PAL of 0.41 ± 0.43 mm after one year following non-surgical periodontal therapy (Baelum *et al.*, 2016). Van der Weijden and Timmerman (2002) in their systematic review on the clinical efficiency of subgingival debridement in patients with chronic periodontitis reported a 0.64 mm attachment gain in PPDs greater than 4 mm (Van der Weijden *et al.*, 2002). The attachment gain in this current study was higher in both treatment groups when comparing to studies utilising conventional non-surgical debridement; however lower than 1.92 mm gain in attachment reported in the Stambaugh *et al.*, (2002). However, study participants in

Stambaugh *et al.*, (2000) were followed for three years post- perioscopy (Stambaugh, 2002). Blue *et al.*, (2013) found that the gain in clinical attachment levels were not related to the use of the perioscope (Blue *et al.*, 2013). In the study by Liao *et al.*, the AL was lower in the group treated with the perioscope (2.9 ± 1.2 mm) as compared to the control group (3.6 ± 1.3 mm); however, these differences were not significant ($p=0.061$) (Liao *et al.*, 2016).

PAL was categorized into PAL 1-3 mm, 4-6 mm, 7-9 mm and 10+ mm and compared between the two groups. The control group had a higher percentage of sites with PAL 1-3 mm at baseline (19.05%) as compared to the test group (16.99%). At three months, the percentage of sites with PAL 1-3 mm increased in both groups, by 28.21% in the control group and by 27.53% in the test group. Very little change in mean percentage of PAL 1-3 mm was seen at twelve months in the control group, with only a 0.5% increase. In the test group, the increase in mean percentage of PAL 1-3 mm, was 4.29%. When examining PALs, for PAL 1-3 mm, the test group had more attachment gain (31.82%) as compared to the control group (28.73%) at twelve months. However, no statistically significant differences were noted between the groups. The study by Mlachkova *et al.*, (2014) utilizing conventional SRD grouped PAL 1-2 mm and found that a significant increase of PAL 1-2 mm ($27.95 \pm 3.88\%$) post therapy (Mlachkova *et al.*, 2014). Badersten *et al.*, (1985) found that probing attachment loss was more frequently detected in sites with initial PPDs ≤ 3.5 mm followed by sites with initial moderate PPDs of 4.0-6.5 mm deep (Badersten *et al.*, 1985c).

For mean percentage of sites with PAL 4-6 mm, the test group recorded a higher percentage at baseline, 3.3% higher than the control group. At three months, in the test group, the mean percentage PAL 4-6 mm reduced by 13.75% as compared to the control group which reduced by 15.71%, suggesting more attachment gain in the control group post-SRD. At twelve months, there were further reductions of mean percentage of PAL 4-6 mm in both groups; with a 0.49% gain in attachment in the control group and a 2.66% gain in attachment in the test group. When considering gains in attachment levels from three months to twelve months, the test group recorded a greater percentage of attachment gain. However, statistical significance was not reported. Gain in clinical attachment level after a 21-month period for PPDs 4-6 mm was 3.64 mm in Stambaugh and Myers (2003) utilizing the perioscope (Stambaugh *et*

al., 2003). Some studies have reported a mean attachment gain of 0.5 mm in PPDs 4-6 mm following non-surgical debridement (Badersten *et al.*, 1984b; Becker *et al.*, 1988; Cercek *et al.*, 1983; Hämmerle *et al.*, 1991; Kaldahl *et al.*, 1988; Lindhe *et al.*, 1982; Sbordone *et al.*, 1990; Sherman *et al.*, 1990). The meta-analysis by Heitz-Mayfield *et al.*, (2005) reported that in PPDs 4-6 mm, SRD resulted in 0.3 mm more attachment gain (Heitz-Mayfield, 2005).

The mean percentage of sites with PAL 7-9 mm in the control group was higher in the control group ($18.20 \pm 12.3\%$) at baseline as compared to the test group ($16.78 \pm 8.9\%$). Post-SRD, in the control group mean percentage of PAL 7-9 mm reduced by 11.5% and 11.65% in the test group. At twelve months further reductions were observed in both groups with the control group reducing a further 0.09% at twelve months and 1.6% in the test group. More attachment gain was observed in the test group at both time intervals with the difference between the groups at twelve months bordering on significance ($p=0.07$). The mean percentage reduction of PAL 7-9% reported post-SRD was much higher in the current study as compared to the study by Mlachkova *et al.*, (2014) who found that for PAL >5 mm, a statistically significant reduction of $9.35 \pm 2.67\%$ occurred post-SRD (Mlachkova *et al.*, 2014). The study by Stambaugh and Myers (2003) reported a 4.67 mm gain in attachment in PPDs greater than 6 mm in subjects treated with perioscopy (Stambaugh *et al.*, 2003). Mean gains in attachment levels for PPDs greater than 7 mm, following conventional non-surgical therapy ranged from 0.5 mm (Hill *et al.*, 1981; Loesche *et al.*, 1991) to 0.84 mm (Lavanchy *et al.*, 1987) in non-molar sites. In molar sites mean attachment levels ranged between a loss of 0.5 mm (Chiew *et al.*, 1991; Nordland *et al.*, 1987) and a gain of 0.84 mm (Kalkwarf *et al.*, 1988).

For percentages of sites with PAL 10+ mm the test group recorded a higher percentage ($2.40 \pm 2.8\%$) as compared to the control group pre-SRD ($2.21 \pm 3.2\%$). Post-SRD, decreases were observed in both groups, with the mean percentage decreasing by 1.35% in the control group as compared to 2.14% in the test group post-SRD. There were further changes in percentages at twelve months with the control group increasing by 0.08% and the test group decreasing even further by 0.03%. One can deduce that more attachment gain was observed in the test group as compared to the control group post-SRD and twelve months.

Even though, the clinical findings suggest that SRD with perioscope group recorded more attachment gain more especially at the twelve-month f/up visit as compared to the SRD- only, gains in PAL were not statistically significant.

3.3.3 Effect of SRD-only and SRD-with perioscope on Gingival Recession

The magnitude of change between PPDs and PALs following non-surgical periodontal therapy is not equal with the difference between these two parameters resulting in gingival recession. At baseline, there was a significant difference in mean recession between the two groups with the test group reporting a higher mean recession (0.90 ± 0.4 mm) as compared to the control group (0.46 ± 0.7 mm). The test group also had a higher mean PAL (5.13 ± 0.6 mm) as compared to the control group (5.09 ± 0.8 mm), although this difference was not significant. When examining the change in gingival recession from baseline to twelve months, the test group had a statistically significant lower mean change in recession (-0.13 ± 0.2 mm) as compared to the control group (-0.5 ± 0.6 mm) ($p=0.01$). The SRD-only group recorded a 0.45 mm increase in mean recession from baseline to three months. In the SRD-with perioscope group, the mean recession only increased from 0.9 mm to 1.0 mm (0.1 mm increase) from baseline to three months. At twelve months, the SRD-only group had a further 0.05 mm increase in mean gingival recession, whereas a 0.03 mm increase was seen in the SRD-with perioscope group. Gingival recession has been reported between 0.13-0.72 mm following non-surgical periodontal debridement (Hughes *et al.*, 1978; Kaldahl *et al.*, 1988). A 0.1 mm change in mean recession from baseline to three months post-RP in subjects with PPDs 1-4 mm was observed in the study by Kaldahl *et al.*, (1988). At twelve months mean change in recession increased to 0.26 mm in this group. For moderate pockets 5-6 mm, mean change in recession was 0.27 mm at three months and increased to 0.44 mm at one year. For deeper pockets >7 mm, mean change in recession was 0.51 mm at three months and increased to 0.72 mm at the one year f/up examination (Kaldahl *et al.*, 1988).

Even though the same instruments were used, the results are indicative that the use of perioscope resulted in less gingival recession. One can speculate that the ability to

directly visualise and remove the subgingival deposits in a less invasive way may result in less trauma to the gingival tissues.

3.3.4 Effect of SRD-only and SRD-with perioscope on Bleeding on Probing

In the 1980s and 1990s in many studies, BOP was examined as a possible predictor of clinical attachment loss (Badersten *et al.*, 1985d, 1990; Claffey *et al.*, 1990; Lang *et al.*, 1986). Joss *et al.* (1994) found that two-thirds of all sites with AL were found in patients that had greater than 30% BOP, while patients with less than 20% had a significantly lower risk of further AL (Joss *et al.*, 1994). Lang *et al.*, (1990) suggested that rather using the presence of BOP, absence of BOP was a more reliable to predict stability (Cobb, 2002; Lang *et al.*, 1990).

For this study population, the control group had a higher mean % of BOP ($60.16 \pm 19.6\%$) as compared to the test group ($48.37 \pm 16.8\%$) before the commencement of treatment. This is suggestive that the control group had more inflammation pre-SRD. Post-SRD resulted in substantial decreases in BOP percentages in both groups. There was more than a 70% reduction in BOP from baseline to three months in both treatment groups; with difference between the groups, bordering but not reaching significance post-SRD. The percentage reduction in the control group was 71% post SRD as compared to 76% in the test group. This study is in line with other studies that report that non-surgical debridement can predictably reduce levels of inflammation (Badersten *et al.*, 1981; Badersten *et al.*, 1984b; Haffajee *et al.*, 1997b; Haffajee *et al.*, 1984; Hämmerle *et al.*, 1991; Heitz-Mayfield *et al.*, 2002; Kalkwarf *et al.*, 1989; Lim *et al.*, 1996; Proye *et al.*, 1982). The percentage reduction in BOP at three months is comparable to other studies where an 80% reduction in BOP were reported at three months post-therapy in the study by Laurell and Pettersson (1988) (Laurell *et al.*, 1988), whereas Hämmerle *et al.*, (1991) reported a 74% decrease in BOP post-therapy (Hämmerle *et al.*, 1991). The results of the current study are similar to Blue *et al.*, (2013) who found that the adjunctive use of the perioscope improved gingival inflammation and bleeding upon probing. The mean change in BOP from baseline to three months was found to be significantly greater in the perioscope sites when compared to the conventional SRD sites ($p=0.036$) (Blue *et al.*, 2013).

Studies by Liao *et al.*, (2016) and Hou (2016) also reported more reductions in BOP with the use of the perioscope; however, no statistically significant differences was found between groups (Hou.Y, 2016; Liao *et al.*, 2016).

The percentage of sites with BOP during the SPT phase of treatment also showed additional decreases in BOP% with the test group having a significantly lower percentage of BOP as compared to the control group at six and nine months. Even though the control group had a higher BOP% at the start of the trial, the percentage reduction in BOP from baseline to twelve months, was higher in the test group (90%) as compared to the control group (80%). At twelve months, mean BOP% in the test group was only 4.63% as compared to 11.95% in the control group and this difference was statistically significant. Cugini *et al.*, (2000) also reported that BOP continued to decrease over the 12 month period following SRD and SPT (Cugini *et al.*, 2000).

Previous studies have suggested that the presence of BOP after non-surgical periodontal therapy can be indicative of residual deposits present in the pockets (Checchi *et al.*, 2009; Colombo *et al.*, 2002). Many studies have supported that instrumentation techniques are not totally effective in removing all calculus and bacteria from the subgingival area (Anderson *et al.*, 1996; Brayer *et al.*, 1989; Buchanan *et al.*, 1987; Caffesse *et al.*, 1986; Dragoo, 1992; Johnson *et al.*, 1989; Nagy *et al.*, 1992; Rabbani *et al.*, 1981; Waerhaug, 1978; Wylam *et al.*, 1993). Four to forty three percent of remnants of plaque and calculus were found after instrumentation in PPDs up to 3 mm (Brayer *et al.*, 1989; Buchanan *et al.*, 1987; Clifford *et al.*, 1999; Gellin *et al.*, 1986; Rabbani *et al.*, 1981). In pocket depths 4-6 mm, 15-38% of treated sites had plaque and calculus (Brayer *et al.*, 1989; Buchanan *et al.*, 1987; Carey *et al.*, 2001; Clifford *et al.*, 1999; Gellin *et al.*, 1986; Kawanami *et al.*, 1988; Rabbani *et al.*, 1981) and in pocket depths greater than 6 mm, 19% to 66% of sites had residual plaque and calculus (Anderson *et al.*, 1996; Brayer *et al.*, 1989; Buchanan *et al.*, 1987; Carey *et al.*, 2001; Clifford *et al.*, 1999; Gellin *et al.*, 1986; Kawanami *et al.*, 1988; Lee *et al.*, 1996; Rabbani *et al.*, 1981).

A direct relationship was established between BOP and subgingival deposits when using endoscopic visualization to view pockets with BOP (Checchi *et al.*, 2009). Wilson *et al.*, (2008), found a statistically significant relationship between subgingival calculus covered with biofilm and inflammation of the pocket wall using periodontal endoscopy.

In more than 60% of the cases, inflammation and increased redness of the pocket epithelium was associated with deposits of subgingival calculus covered with biofilm (Wilson *et al.*, 2008a). It was also reported that elimination of histological signs of chronic inflammation occurred six months after endoscopic debridement (Wilson *et al.*, 2008b).

The study by Michaud *et al.*, (2007) did not find significant improvements in calculus removal in multi-rooted teeth with the use of the perioscope. This study examined 70 teeth in total. Teeth were extracted and residual calculus was determined using stereomicroscopy and digital image software (Michaud *et al.*, 2007). Geisinger *et al.*, (2007) examined whether the use of the perioscope for SRD resulted in decrease in residual calculus as compared to SRD-alone on single-rooted teeth. Fifteen subjects with 50 tooth pairs were included in the study with each tooth pair randomized to receive SRD with or without the perioscope. Their study reported that there was 2.14% ($p < 0.001$) more residual calculus at the SRD-only sites as compared to the SRD-with perioscope sites. This study concluded that significantly less residual calculus was found with the use of the perioscope at deeper probing depths as compared to conventional SRD (Geisinger *et al.*, 2007). For the current study, the lower BOP in the test group could suggest that the ability to directly visualise the root surface during debridement with an endoscope resulted in less residual calculus and biofilm and therefore less BOP.

For the present study, the perioscope was not used to check sites with BOP post-SRD or during the SPT appointments. This did not form part of the study protocol. The remaining pockets with BOP in both groups may be suggestive of the presence of residual calculus and associated inflammation. Residual calculus may contribute to the reinfection of the pocket. A high correlation has been described between percent of residual calculus and pocket depth, with pocket depths greater than 3 mm more difficult to manage with traditional SRD (Rabbani *et al.*, 1981).

3.3.5 Effects of SRD-only and SRD-with perioscope on the Plaque Index

Meticulous supragingival plaque removal during the treatment and post-treatment phase has been well established for all forms of periodontal treatment. Several studies have suggested that although supragingival plaque control can lead to some decrease in periodontal inflammation, more reduction is achieved following subgingival debridement (Badersten *et al.*, 1981; Badersten *et al.*, 1984b; Cercek *et al.*, 1983; Lim *et al.*, 1996; McNabb *et al.*, 1992; Westfelt *et al.*, 1998). For the current study, the mean percentage PI was higher in the control group pre-SRD. Post-therapy showed improvements in plaque control in both groups similar to findings in other studies (Badersten *et al.*, 1984b; Cercek *et al.*, 1983; Magnusson *et al.*, 1984; Nordland *et al.*, 1987). Mean PI% continued to improve in the test group at six, nine and twelve months. The control group also had similar results with slight increases in PI% at nine months. A statistically significant lower mean PI% was reported in the test group as compared to the control group at nine and twelve months. Low supragingival plaque levels can contribute to the maintenance of periodontal health and meticulous plaque control can reduce inflammation especially around the marginal gingiva. The lower PI% could have contributed to the lower BOP% in the test group.

The higher mean PI in the control group could be related to the higher mean PPD in this group. Studies have found that mean pocket depths can decrease with effective plaque control (Kho *et al.*, 1985), with more reductions observed with professional plaque and calculus removal (Glavind, 1977; Kho *et al.*, 1985). However, in deep pockets, supragingival plaque does not have any obvious effects on clinical parameters (Beltrami *et al.*, 1987).

One of the limitations of the PI used was that the index recorded the presence or absence of plaque, rather than the actual quantity of plaque present.

3.3.6. The Role of Supportive Periodontal Maintenance

The role of supportive periodontal maintenance in maintaining and stabilizing the periodontium must also be recognised. Axelsson and Lindhe (1981) emphasised the role of a supervised supportive periodontal maintenance following active periodontal therapy. In their study, it was described that patients who followed a maintenance programme were able to maintain “excellent oral hygiene standards and unaltered attachment levels”. Patients who did not follow through with the supervised maintenance programme, showed signs of recurrent periodontitis in the follow-up appointments (Axelsson *et al.*, 1981). Axelsson *et al.*, (2004) monitored tooth loss, AL and caries during a 30-year period in a private dental office. The incidence of caries, periodontal disease and tooth loss were reported to be very low in this study population (Axelsson *et al.*, 2004). Kerr (1981) also reported the likelihood of recurrence of disease for those patients not in a professional maintenance programme. Forty-five percent of those patients presented with periodontal conditions similar to their status before treatment (Kerr, 1981). Signs of recurrent disease was observed in those patients who did not continue with regular supportive periodontal maintenance in the retrospective study by Becker *et al.*, (1984) (Becker *et al.*, 1984). Studies have concurred that periodontal maintenance is important and can reduce risk variables (Chambrone *et al.*, 2010; Costa *et al.*, 2012a; Fardal, 2011; Wilson *et al.*, 1984).

SPT is employed to reduce the likelihood of reinfection and hinder the progression of the disease (Manresa *et al.*, 2018). The study by De Wet *et al.*, found that patients receiving supportive periodontal maintenance were able to maintain their periodontal condition (De Wet *et al.*, 2018). Other studies have demonstrated that higher rates of tooth loss can occur in the absence of regular SPT (Kocher *et al.*, 2000; Wilson *et al.*, 1984). All participants in the current study were placed in a three monthly SPT programme and were compliant during the study period. Improvements in clinical outcomes were seen in both groups at the twelve-month re-evaluation appointment, following three-month SPT appointments.

3.4. Conclusion

The preferred outcome of periodontal therapy as described by the AAP is “significant reduction of clinical signs of gingival inflammation, reduction of probing depths and stabilization of clinical detectable plaque to a level compatible with gingival health” (AAP, 2000). Our results support the effectiveness of both treatment modalities in reducing clinical parameters post-SRD and at twelve months; however, some improvement in clinical outcomes were obtained with the use of the perioscope for some of the clinical parameters. Mean PPD reduced in both groups post-SRD and at twelve months with a statistically significantly lower mean PPD recorded in the test group at twelve months. Reductions in percentages of sites with PPDs 1-3 mm, 4-6 mm and 10+ mm were observed in both groups; however, in the deeper sites, that is ,PPDs 7-9 mm, the percentages of sites were found to be significantly lower in the SRD-with perioscope group suggesting that the perioscope may be a useful tool to visualise and debride deeper pockets. In addition, when examining PPDs 1-4 mm, the SRD-with perioscope had a significantly higher percentage of PPDs in this category ($95.62 \pm 4.6\%$) as compared to the control group ($89.87 \pm 7.9\%$). However, more RCTs, trials with larger study populations are required to test the efficacy of the periodontal endoscope as compared to conventional non-surgical debridement.

The use of the perioscope resulted in more attachment gain; however, the difference between the two groups were not statistically significant. More attachment gain was observed in sites with PAL 7-9 mm and 10+ mm in the SRD-with perioscope group with results bordering on significance. This calls for more controlled trials with larger sample sizes to analyse the effect of the perioscope on PALs when compared to conventional treatment.

The amount of gingival recession recorded with the use of the perioscope was lower than conventional SRP. It can be a suggested method of debridement especially in aesthetic zones and in patients with thin biotype where gingival recession should be kept to its minimal.

The test group had better oral hygiene than the control group as reflected by the statistically significant lower PI% at twelve months. Good supragingival plaque control could have contributed to the lower BOP% in this group. The lower BOP% in the test

group could also be result of less residual calculus and biofilm on the root surfaces due to the use of the direct visualisation technique. However, it will be beneficial for future studies to utilize the perioscope post-treatment to determine the presence of residual calculus.

It is also important to consider that even though some of the differences in clinical parameters were statistically significant with the use of periodontal endoscope, the differences were small. The SRD-only group had higher mean PPDs, more inflammation and poorer oral hygiene as compared to the SRD-with perioscope group at the start of the trial, even though the differences in mean PPDs and BOP were not statistically significant. More RCTs are required to test the efficacy of the use of the periodontal endoscope as an adjunct to non-surgical periodontal therapy.

It is often difficult to determine the “end point” following conventional non-surgical debridement. Even experienced operators may think that the root surface is smooth and free of deposits after instrumentation; however burnished calculus and root anomalies make it difficult to determine if the “end point” is achieved. Periodontal endoscopy allows the clinician to directly visualise the root surface, identify calculus and remove deposits non-surgically. However, this technique does have its limitations. It has a high learning curve and needs the operator to be trained to identify the field of vision as well as landmarks. It requires that you become proficient with your non-dominant hand. Severity of inflammation can also make it difficult to view and access root surfaces. Difficulties can be experienced when accessing distal furcations of maxillary molars, narrow furcations as well as Class III furcations. In addition, access may be limited in areas where the roots are in close proximity and even in sites where restorations are over-contoured (Harrel *et al.*, 2015).

Despite these limitations, an option to treat periodontal disease in a less invasive way may be advantageous. Developments in technology may result in a simpler way to use direct visualization technology.

CHAPTER 4

MICROBIOLOGICAL ANALYSIS

This chapter describes the results of the microbiological tests for the test and control groups at baseline(pre-SRD), three(post-SRD) and twelve months. The method used for bacterial sampling and the techniques employed by Hain Lifescience, Nehren, Germany, is explained in detail in Chapter 2.

The percentage of each pathogen at each level is reported and compared at each time point. The prevalence of each pathogen below threshold (level 0) and above threshold (levels 1,2,3,4) were computed at baseline (pre-SRD), three months(post-SRD) and at twelve months for both the test and control groups.

4. 1 Analyses of Eleven Pathogens

Table 4.1 provides baseline, three- and twelve-month statistical data obtained from using the Mann Whitney *U* tests for the individual pathogens pre-SRD. The entire set is ordered, and a rank is then assigned to each case, returned to the control and test groups and then summed and averaged. The mean rank is this average. The mean rank allows comparison of each pathogen between the two groups (similar to the T-test).

Table 4. 1 Mean Rank, U, z-value and p-value for the eleven pathogens at baseline (pre-SRD) for control and test groups at baseline, three and twelve months

	Control n=19	Test n=19	Test Statistics		
Pathogen	Mean Rank	Mean Rank	<i>U</i>	z-value	p- value*
<i>A. actinomycetemcomitans</i>					
Baseline	19.55	19.45	179.50	-0.046	1.0
Three months	19.45	19.55	179.50	-0.055	1.0
Twelve months	19.42	19.58	179.00	-0.069	1.0
<i>P. gingivalis</i>					
Baseline	18.21	20.79	156.00	-0.784	0.46
Three months	18.11	20.89	154.00	-0.857	0.43
Twelve months	18.32	20.68	158.00	-0.751	0.48
<i>T. forsythia</i>					
Baseline	19.95	19.05	172.00	-0.367	0.83

Three months	17.92	21.08	150.50	-1.018	0.40
Twelve months	20.26	18.74	166.00	-0.467	0.64
<i>T. denticola</i>					
Baseline	22.42	16.58	125.00	-1.821	0.08
Three months	18.32	20.68	158.00	-0.713	0.50
Twelve months	21.32	17.68	146.00	-1.097	0.29
<i>P. intermedia</i>					
Baseline	18.13	20.87	154.50	-0.925	0.38
Three months	18.74	20.26	166.00	-0.530	0.57
Twelve months	19.29	19.71	176.50	-0.173	1.0
<i>P. micros</i>					
Baseline	19.26	19.74	176.00	-0.150	1.0
Three months	20.16	18.84	168.00	-0.413	0.72
Twelve months	21.66	17.34	139.50	-1.254	0.23
<i>F. nucleatum</i>					
Baseline	19.53	19.47	180.00	-0.016	1.0
Three months	18.58	20.42	163.00	-0.719	0.64
Twelve months	19.76	19.24	175.50	-0.166	1.0
<i>C. rectus</i>					
Baseline	22.29	16.71	127.50	-1.678	0.09
Three months	19.08	19.92	172.50	-0.252	0.83
Twelve months	19.71	19.29	176.50	-0.122	0.95
<i>E. nodatum</i>					
Baseline	18.26	20.74	157.00	-0.734	0.51
Three months	19.95	19.05	172.00	-0.265	0.83
Twelve months	18.11	20.89	154.00	-0.853	0.45
<i>E. corrodens</i>					
Baseline	20.50	18.50	161.50	-0.874	0.4
Three months	18.16	20.84	155.00	-1.0	0.43
Twelve months	19.00	20.00	171.00	-0.308	0.80
<i>Capnocytophaga sp.</i>					
Baseline	18.03	20.97	152.50	-0.874	0.39
Three months	18.55	20.45	162.50	-0.547	0.60
Twelve months	18.21	20.79	156.00	-0.764	0.46

*N<20, exact significance reported

4.1.1 Pathogen Numbers at Baseline (Pre-SRD)

The mean rank for *P. gingivalis* was 20.79. This indicates that *P. gingivalis* in the test group appeared more frequently in the higher ranked positions (implying higher numbers) when compared to the control group (mean rank 18.21). This was also true for the mean ranks of *P. intermedia*, *P. micros*, *E. nodatum* and *Capnocytophaga sp.* In the control group, *A. actinomycetemcomitans*, *T. forsythia*, *T. denticola*, *F. nucleatum*, *C. rectus* and *E. corrodens* had higher mean ranks or higher numbers as compared to the test group. However, these differences in mean ranks between groups at baseline were not statistically significant ($p>0.05$). Interestingly, *T. denticola* and *C. rectus* were found in higher numbers in the control group with the differences in mean ranks approaching but not reaching statistical significance ($p=0.08$; 0.09 respectively).

4.1.2 Pathogen Numbers at Three months (Post SRD)

No statistically significant differences ($p>0.05$) were observed between mean ranks of the test and control groups post SRD. When examining mean rank data between groups post-SRD, the test group had nine of the eleven pathogens detected with higher numbers when compared to the control group. *P. gingivalis*, *P. intermedia* and *Capnocytophaga sp.* remained higher in the test group; however, increases in numbers of *A. actinomycetemcomitans*, *T. forsythia*, *T. denticola*, *F. nucleatum*, *C. rectus* and *E. corrodens* were observed in test group with improvements in numbers of these pathogens seen in the control group. The numbers of both *T. denticola* and *C. rectus* in the control group at baseline were much higher than the test group and bordered on significance. Post-SRD saw decreases in numbers of these two pathogens with higher numbers now observed in the test group. The numbers of *P. micros* and *E. nodatum* improved in the test group post-SRD and was now detected with higher numbers in the control group. (Table 4.1)

4.1.3 Pathogen Numbers at Twelve Months

At twelve months, although changes in mean ranks of the eleven pathogens were observed, there were no statistically significant differences observed between the two groups ($p>0.05$).

At baseline, the test group had 5 pathogens namely *P. gingivalis*, *P. intermedia*, *P. micros*, *E. nodatum* and *Capnocytophaga* sp. with higher mean ranks. The control group had six pathogens with higher mean ranks namely *A. actinomycetemcomitans*, *T. forsythia*, *T. denticola*, *F. nucleatum*, *C. rectus* and *E. corrodens*.

Mean ranks changed post-SRD with nine pathogens (*P. gingivalis*, *P. intermedia*, *Capnocytophaga* sp., *A. actinomycetemcomitans*, *T. forsythia*, *T. denticola*, *F. nucleatum*, *C. rectus* and *E. corrodens*) having higher mean ranks in the test group as compared to the control group post-SRD. The control group had two pathogens namely *P. micros* and *E. nodatum* with higher mean ranks post-SRD.

At twelve months, six pathogens had higher mean ranks in the test group as compared to the control group. The numbers of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *E. corrodens* and *Capnocytophaga* sp. still remained higher in the test group as compared to the control group; with *E. nodatum* also increasing in numbers in this group. There was improvement in bacterial numbers of *T. forsythia*, *T. denticola*, *F. nucleatum* and *C. rectus* in the test group with these pathogens observed at higher ranks in the control group. *P. micros* remained higher in the control group. At twelve months, six of the eleven pathogens were found in higher numbers in the test group, and five in the control group. The test group had a reduction of number of pathogens from post-SRD to twelve months from nine to six pathogens, whilst the control group showed an increase in the number of pathogens from two at post-SRD to five at twelve months. (Table 4.1)

4.2 Analysis of individual pathogens: Prevalence and percentage of pathogens at baseline (pre-SRD), three months (post-SRD) and twelve months

Prevalence of each pathogen at below detectable levels (level 0) and above detectable levels (level 1,2,3,4) were compared at each time point. Levels were grouped into “below threshold” and “above threshold”.

Bacterial results received included the detected level of each pathogen, that is either level 0,1,2,3 or 4. Using these results, the percentage of the pathogen at each level was calculated pre-SRD, post-SRD and twelve months for both groups. Increases, decreases or no changes in percentages at each level is also described.

4.2.1 *Aggregatibacter (Actinobacillus) actinomycetemcomitans*

4.2.1.1 Prevalence of *A. actinomycetemcomitans*

There were no differences in the prevalence of *A. actinomycetemcomitans* between the test and control group pre-SRD, post-SRD and at twelve months. Only 3 of the 19 participants (16%) had detectable levels of *A. actinomycetemcomitans* and 16 of the 19 (84%) participants had *A. actinomycetemcomitans* below threshold for both control and test groups (Table 4.2)

Slight decreases in prevalence of *A. actinomycetemcomitans* were observed in both groups post-SRD, with 17 of the 19 participants (89%) having *A. actinomycetemcomitans* below detectable levels and only 2 of the 19 having *A. actinomycetemcomitans* above threshold levels. At twelve months, the prevalence of *A. actinomycetemcomitans* returned to pre-SRD levels for both groups (Table 4.2).

Table 4. 2 Prevalence of *A. actinomycetemcomitans* baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>A. actinomycetemcomitans</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	16 (84%)	3 (16%)	n=19 (100%)	16 (84%)	3 (16%)	n=19 (100%)
Three Months	17 (89%)	2 (11%)	n=19 (100%)	17 (89%)	2 (11%)	n=19 (100%)
Twelve Months	16 (84%)	3 (16%)	n=19 (100%)	16 (84%)	3 (16%)	n=19 (100%)

4.2.1.2 Analysis of Percentage of *A. actinomycetemcomitans* at each level pre-SRD, post-SRD and at twelve months

Figure 4.1 and Table 4.3 represents the percentages at baseline (pre-SRD), three months (post-SRD) and twelve months.

- For level 0, which denotes “*bacterial load under cut off*” (“*A. actinomycetemcomitans*<10₃”), *A. actinomycetemcomitans* increased in both control and test groups post-SRD and returned to baseline percentage at twelve months.
- For “*bacterial load at cut off*” (“*A. actinomycetemcomitans*:10₃”) or level 1, *A. actinomycetemcomitans* was at 0% at baseline and three months for both groups. However, there was a marginal increase in the control group to 5.26% at twelve months, whereas the percentage remained at 0% in the test group for this level of *A. actinomycetemcomitans*.
- *A. actinomycetemcomitans* was recorded at 0% for “*Increased Bacterial load*” (*A. actinomycetemcomitans* <10₄) or level 2 in the control group at all three time points. In the test group, a 5.3% for level 2 was recorded pre-SRD; however, this was reduced to 0% at three and twelve months.

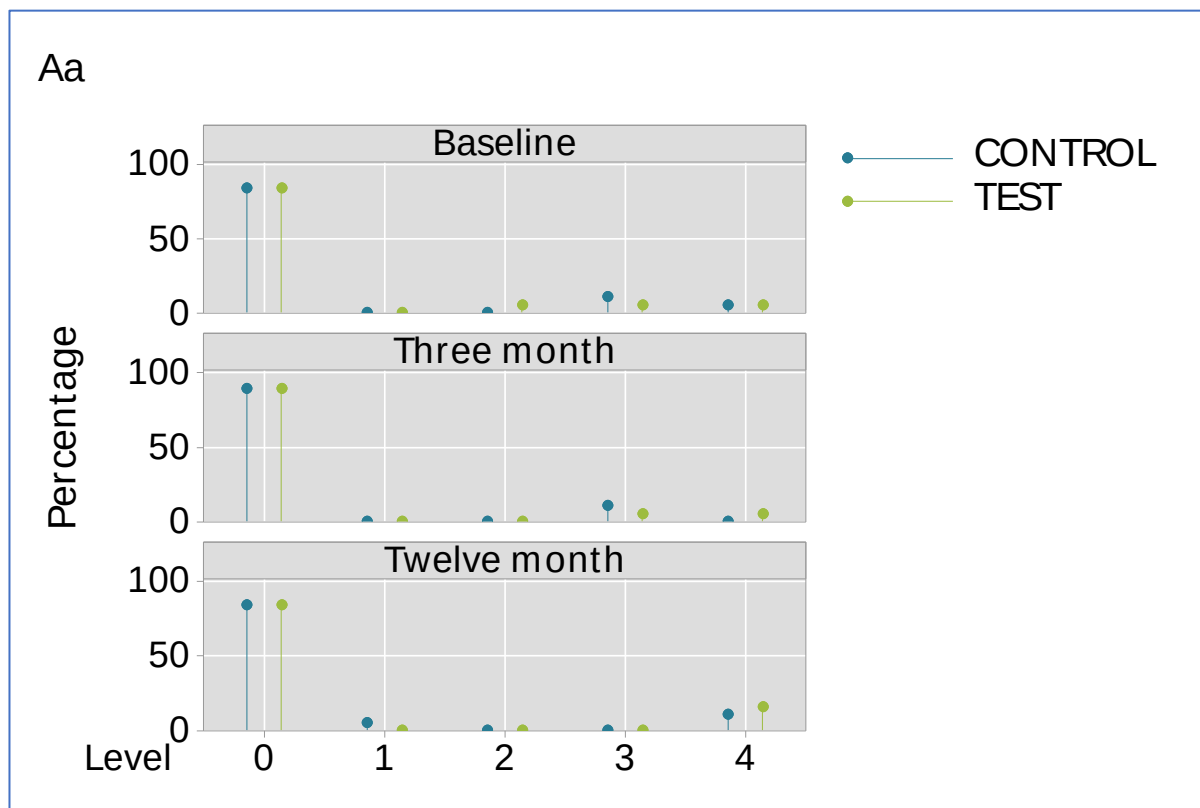
- Both the groups showed no changes in percentage of *A. actinomycetemcomitans* at level 3 or “++High bacterial load” (*A. actinomycetemcomitans*<10⁵) post -SRD. At twelve months, both groups reported a 0% of *A. actinomycetemcomitans* at this level.
- For Level 4 or “+++ Extremely high bacterial load” (*A. actinomycetemcomitans*> 10⁵), the percentage in the control group decreased from 5.26% pre-SRD to 0% at three months (post-SRD) and then increased to 10.53% at twelve months. In the test group, the percentage of *A. actinomycetemcomitans* at level 4 remained the same at 5.26% post SRD and then increased to 15.79% at twelve months.

Table 4. 3 Percentage of *A. actinomycetemcomitans* at each level at baseline (pre-SRD), three months (post-SRD) and twelve months including increase, decrease or no change in percentage

Groups	Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage twelve months	↑/↓/0*
<i>A. actinomycetemcomitans</i>						
Control	0	84.21	89.47	↑	84.21	↓
Test	0	84.21	89.47	↑	84.21	↓
Control	1	0.0	0.00	0	5.26	↑
Test	1	0.0	0.00	0	0.00	0
Control	2	0.00	0.00	0	0.00	0
Test	2	5.26	0.00	↓	0.00	0
Control	3	10.53	10.53	0	0.00	↓
Test	3	5.26	5.26	0	0.00	↓
Control	4	5.26	0.00	↓	10.53	↑
Test	4	5.26	5.26	0	15.79	↑

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 1 A comparison of percentages for *A. actinomycetemcomitans* at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.2.2 *P. gingivalis*

4.2.2.1 Prevalence of *P. gingivalis*

At baseline (pre-SRD), the test group has a higher prevalence of *P. gingivalis* above threshold (53%) as compared to the control group (42%). Nine of the 19 participants (47%) in the test group had below detectable levels of *P. gingivalis*, compared to 11 of the 19 (58%) in the control group (Table 4.4).

Post-SRD, the number of participants with levels below and above threshold remained the same in the test group. In the control group, the prevalence of *P. gingivalis* increased to 12 of the 19 participants having *P. gingivalis* below detectable levels and to 7 of the 19 having *P. gingivalis* at above threshold levels (Table 4.4).

At twelve months, no changes in prevalence was detected in the test group.

The control group had 13 of the 19 (68%) participants with *P. gingivalis* at below detectable levels and 6 of the 19 (32%) above detectable levels.

Table 4. 4 Prevalence of *P. gingivalis* at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>P. gingivalis</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	11 (58%)	8(42%)	n=19 (100%)	9(47%)	10 (53%)	n=19 (100%)
Three Months	12 (63%)	7 (37%)	n=19 (100%)	9 (47%)	10 (53%)	n=19 (100%)
Twelve Months	13 (68%)	6 (32%)	n=19 (100%)	9 (47%)	10 (53%)	n=19 (100%)

4.2.2.2 Analysis of Percentage of *P. gingivalis* at each level pre-SRD, post-SRD and at twelve months

The percentage of *P. gingivalis* for each level at each time point is illustrated in Table 4.5. Figure 4.2 provides a comparison of the percentages for each level of *P. gingivalis* pre-SRD, post-SRD and at twelve months.

The following changes in percentages are noted:

- The percentage of *P. gingivalis* at level 0 or “bacterial load under cut off” (*P. gingivalis*=<10₄) increased at three months and twelve months in the control group. The test group reported no change in percentage for level 0 of *P.*

gingivalis post- SRD and remained at 47.37%; and then at twelve months, an increase in percentage of *P. gingivalis* to 52.63% was observed.

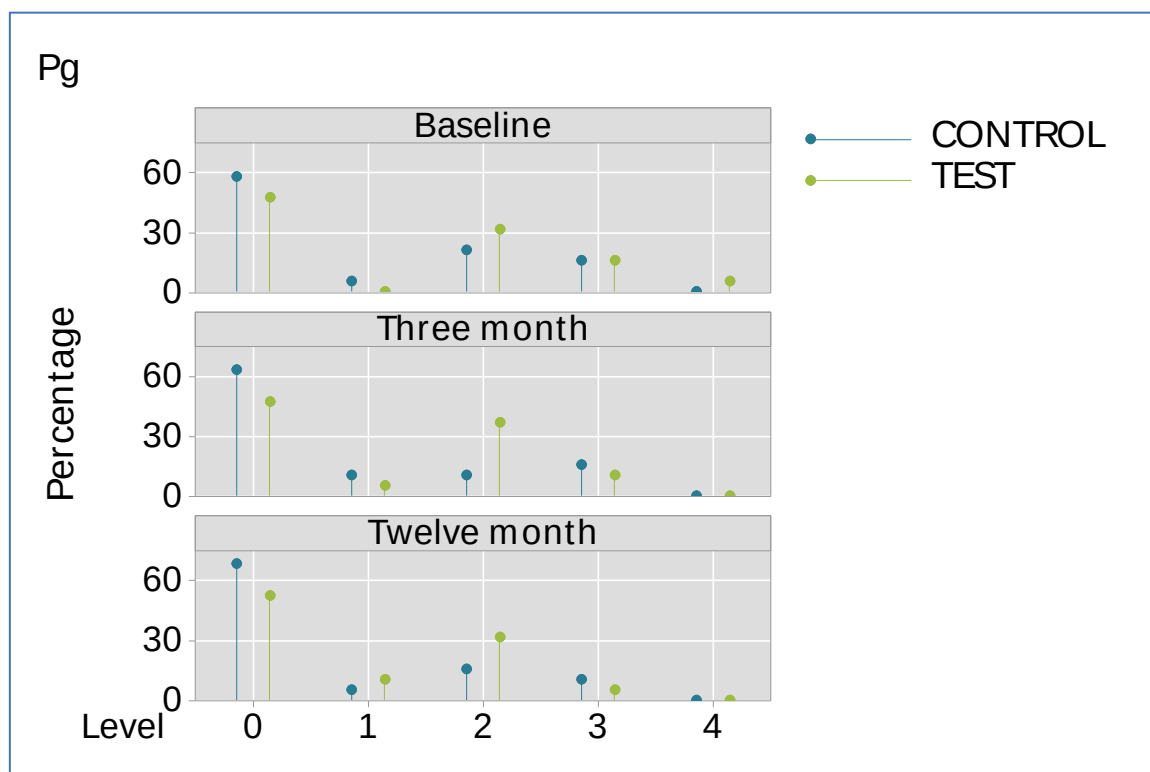
- For level 1 or “*Bacterial load at cut off*” (*P. gingivalis*= 10^4), the percentage of *P. gingivalis* increased from 5.26% to 10.53% in the control group post- SRD and then decreased to the baseline percentage (5.26%) at twelve months. In the test group, there were increases in percentage of level 1 of *P. gingivalis* post-SRD (5.26%) and then a further increase in percentage to 10.53% at twelve months.
- The control group showed decreases in percentages of *P. gingivalis* at level 2 or “*Increased Bacterial load*” (*P. gingivalis*= $<10^5$) post -SRD and an increase in percentage of *P. gingivalis* at level 2 at twelve months. The test group had an increase in percentage of level 2 of *P. gingivalis* post-SRD and at twelve months a decrease was observed and the percentage of *P. gingivalis* at level 2 returned to the pre-SRD percentage (31.58%).
- No changes in percentage of *P. gingivalis* at level 3 “*High bacterial load*” (*P. gingivalis* = $<10^6$) were observed post-SRD for the control group (15.79%). At twelve months, however, the percentage of *P. gingivalis* at level 3 decreased to 10.53%. In the test group, decreases in percentage of level 3 *P. gingivalis* were observed post-SRD (10.53%) and a further decrease in percentage was noted at twelve months (5.26%).
- There were no observations of level 4 or “*Extremely high bacterial load*” (*P. gingivalis* = $>10^6$) for the control group for each of the time points. The percentage of *P. gingivalis* at level 4 in the test group was 5.26% at baseline and this decreased to 0% post-SRD and twelve months.

Table 4. 5 Percentage of *P. gingivalis* at each level at baseline (pre-SRD), three months (post-SRD) and twelve months including increase, decrease or no change in percentage

Groups	Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage twelve months	↑/↓/0*
<i>P. gingivalis</i>						
Control	0	57.89	63.16	↑	68.42	↑
Test	0	47.37	47.37	0	52.63	↑
Control	1	5.26	10.53	↑	5.26	↓
Test	1	0.0	5.26	↑	10.53	↑
Control	2	21.05	10.53	↓	15.79	↑
Test	2	31.58	36.84	↑	31.58	↓
Control	3	15.79	15.79	0	10.53	↓
Test	3	15.79	10.53	↓	5.26	↑
Control	4	0.00	0.00	0	0.00	0
Test	4	5.26	0.00	↓	0.00	0

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 2 A comparison of percentages for *P. gingivalis* at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.2.3 *T. forsythia*

4.2.3.1 Prevalence of *T. forsythia*

The prevalence of *T. forsythia* at below threshold level was low (5%) for both groups pre-SRD. 18 of the 19 participants (95%) in both groups had detectable levels of *T. forsythia* (Table 4.6).

No changes in prevalence was noted post-SRD in both groups. At twelve months, an increase in prevalence below detectable levels was seen for the test group with 3 of the 19 participants (16%) having *T. forsythia* at below threshold levels. In the control group, all 19 participants had levels above threshold.

Table 4. 6 Prevalence of *T. forsythia* at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>T. forsythia</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	1 (5%)	18 (95%)	n=19 (100%)	1 (5%)	18 (95%)	n=19 (100%)
Three Months	1 (5%)	18 (95%)	n=19 (100%)	1 (5%)	18 (95%)	n=19 (100%)
Twelve Months	0 (0%)	19 (100%)	n=19 (100%)	3 (16%)	16 (84%)	n=19 (100%)

4.2.3.2 Analysis of Percentage of *T. forsythia* at each level pre-SRD, post-SRD and at twelve months

Table 4.7 presents the percentage of *T. forsythia* at each level pre-SRD; post- SRD and at twelve months.

The following changes in percentage of *T. forsythia* was noted (Figure 4.3)

- the percentage for level 0 or “*bacterial load under cut-off* “(*T. forsythia* =<10₄) remained at 5.26% for both groups post-SRD. At twelve months, a 0% of *T. forsythia* at level 0 was recorded in the control group, however in the test group, the percentage of *T. forsythia* at level 0 increased to 15.79% indicating a greater percentage for “*bacterial load under cut off*” for the test group.
- There were no recorded percentages (0%) for *T. forsythia* at level 1 or “*Bacterial load at cut off* “(*T. forsythia* =10₄) for the test group for each of the time points. For the control group, 0% of *T. forsythia* at level 1 was recorded at baseline and three months and an increase to 5.26% was seen at twelve months.

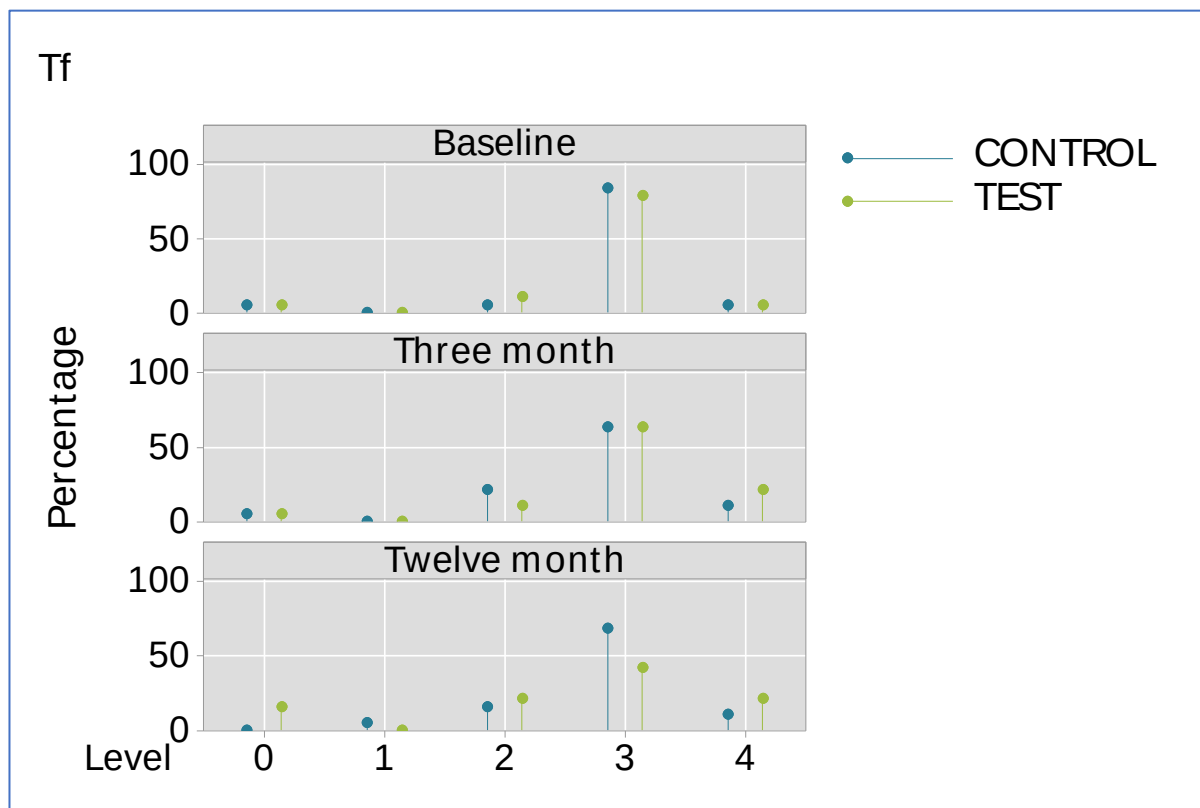
- In the control group, the percentage of *T. forsythia* at level 2 or “*Increased Bacterial load*” (*T. forsythia* = $<10^5$) increased from 5.26% pre-SRD to 21.05% post SRD. At twelve months, there was a decrease in percentage of *T. forsythia* at level 2 (15.79%). In the test group, percentage of *T. forsythia* at level 2 remained unchanged (10.53%) pre and post SRD. However, at twelve months, the percentage of *T. forsythia* at this level increased to 21.05%.
- The percentage of *T. forsythia* at level 3 or “*High bacterial load*” (*T. forsythia* = $<10^6$) decreased from 84.21% pre-SRD to 63.16% post-SRD in the control group. However, at twelve months there was a slight increase to 68.42%. The test group, however had decreases in percentage of *T. forsythia* at level 3 post-SRD (63.16%) and at twelve months (42.11%).
- There were increases in percentage for level 4 or “*Extremely high bacterial load*” (*T. forsythia* = $>10^6$) for both groups post-SRD and this percentage remained the same at twelve months.

Table 4. 7 Percentage of *T. forsythia* at each level baseline (pre-SRD), three months (post-SRD) and at twelve months including increase, decrease or no change in percentage

Groups	Rank/ Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage twelve months	↑/↓/0*
<i>T. forsythia</i>						
Control	0	5.26	5.26	0	0.00	↓
Test	0	5.26	5.26	0	15.79	↑
Control	1	0.00	0.00	0	5.26	↑
Test	1	0.00	0.00	0	0.00	0
Control	2	5.26	21.05	↑	15.79	↓
Test	2	10.53	10.53	0	21.05	↑
Control	3	84.21	63.16	↓	68.42	↑
Test	3	78.95	63.16	↓	42.11	↓
Control	4	5.26	10.53	↑	10.53	0
Test	4	5.26	21.05	↑	21.05	0

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 3 A comparison of percentages for *T. forsythia* at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.2.4 Treponema denticola

4.2.4.1 Prevalence of *T. denticola*

There were minimal differences between the groups for prevalence of *T. denticola* pre-SRD for levels below and above threshold. The control group had all 19 participants at levels above threshold and the test group having 18 of the 19 participants (95%) at this level with only 1 participant below detectable levels for *T. denticola*. (Table 4.8).

Post-SRD, both the test and control groups had 2 of the 19 participants (11%) having below detectable levels of *T. denticola* and 17 of the 19 above threshold levels (89%) At twelve months, the prevalence of *T. denticola* below detectable levels increased in the test group from 2 to 5 of the 19 participants (26%) and only 14 of the 19 (74%) had

T. denticola at levels above threshold. No changes in prevalence were observed in the control group from three to twelve months (Table 4.8).

Table 4. 8 Prevalence of *T. denticola* at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>T. denticola</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	0 (0%)	19 (100%)	n=19 (100%)	1 (55)	18 (95%)	n=19 (100%)
Three Months	2 (11%)	17 (89%)	n=19 (100%)	2 (11%)	17 (89%)	n=19 (100%)
Twelve Months	2 (11%)	17 (89%)	n=19 (100%)	5 (26%)	14 (74%)	n=19 (100%)

4.2.4.2 Analysis of Percentage of *T. denticola* at each level pre-SRD, post-SRD and at twelve months

Table 4.9 presents the percentage of *T. denticola* at each level pre-SRD; post- SRD and at twelve months.

The following changes in percentage of *T. denticola* were observed (Figure 4.4):

- The percentage of *T. denticola* at level 0 or “bacterial load under cut off” (*T. denticola* =<10₄) increased to 10.53% post -SRD in the control group and remained at this percentage at twelve months. In the test group, no changes in percentage of *T. denticola* at level 0 was observed post SRD (5.26%); but an increase to 10.53% was seen at twelve months.

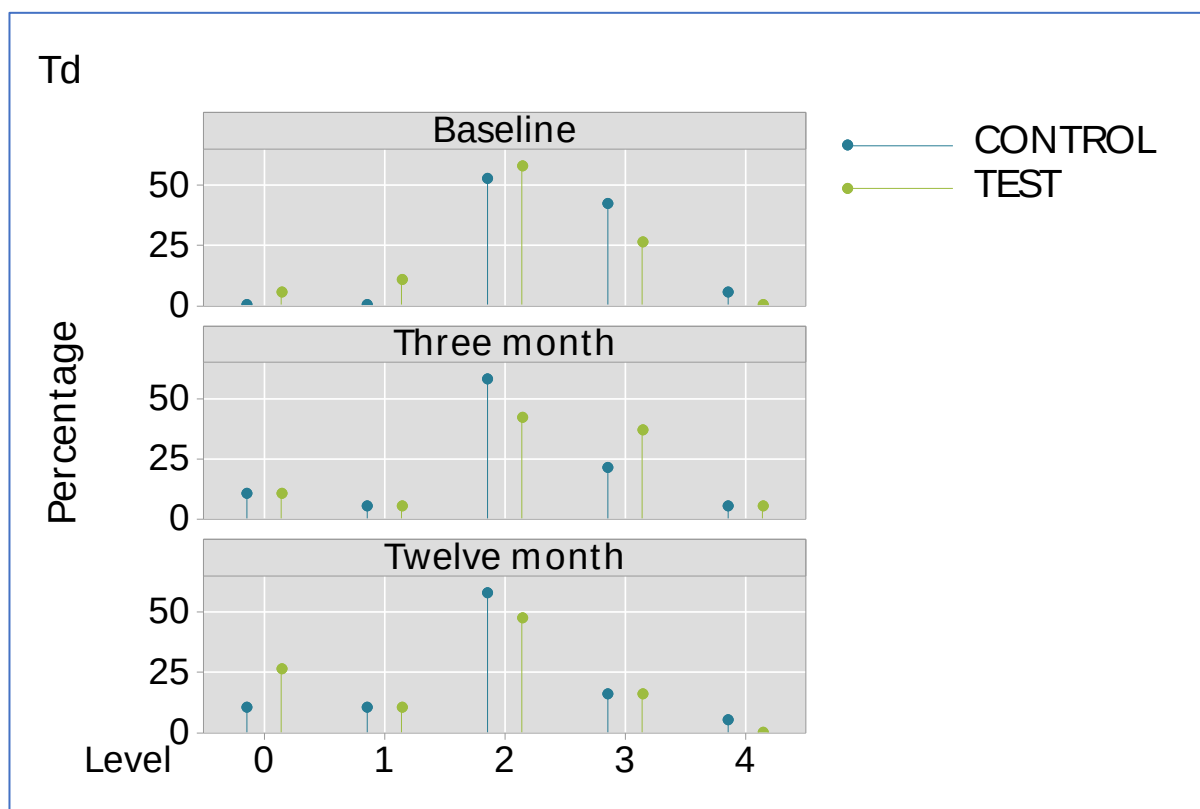
- For level 1 or “*Bacterial load at cut off*” (*T. denticola* = 10^4), the percentage of *T. denticola* at this level, increased from 0% to 5.26% in the control group post-SRD and increased further to 10.53% at twelve months. In the test group, the percentage of *T. denticola* for level 1 decreased from 10.53% to 5.26% post-SRD and then increased to the baseline percentage of 10.53% at twelve months.
- The percentage of *T. denticola* at level 2 or “*increased Bacterial load*” (*T. denticola* = $<10^5$), increased post SRD in the control group and this percentage (57.89%) remained unchanged at twelve months. In the test group, a decrease in percentage of *T. denticola* at level 2 was noted (57.89% to 42.11%) post SRD and then an increase to 47.37% at twelve months.
- For level 3 or “*++ High bacterial load*” (*T. denticola* = $<10^6$), the percentage of *T. denticola* decreased from 42.11% to 21.05% at three months and then decreased even further to 15.79% at twelve months in the control group. In the test group, an increase in percentage of *T. denticola* at level 3 occurred post-SRD (26.32% to 36.84%); however, at twelve months a decrease in “*high bacterial load*” to 15.79% was recorded for the test group.
- *T. denticola* increased to 5.26% for level 4 or “*Extremely high bacterial load*” (*T. denticola* = $>10^6$) in the test group post-SRD and decreased to 0% at twelve months. In the control group the percentage of *T. denticola* for level 4 remained unchanged at 5.26% for all three time points.

Table 4. 9 Percentage of *T. denticola* at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	*Rank	Percentage % at Baseline	Percentage at three months	↑/↓/0*	Percentage at twelve months	↑/↓/0*
<i>T. denticola</i>						
Control	0	0.0	10.53	↑	10.53	0
Test	0	5.26	5.26	0	10.53	↑
Control	1	0.00	5.26	↑	10.53	↑
Test	1	10.53	5.26	↓	10.53	↑
Control	2	52.63	57.89	↑	57.89	0
Test	2	57.89	42.11	↓	47.37	↑
Control	3	42.11	21.05	↓	15.79	↓
Test	3	26.32	36.84	↑	15.79	↓
Control	4	5.26	5.26	0	5.26	0
Test	4	0.0	5.26	↑	0.00	↓

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 4 A comparison of percentages for *T. denticola* at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.2.5 *P. intermedia*

4.2.5.1 Prevalence of *P. intermedia*

The prevalence of *P. intermedia* in both groups was higher at levels below threshold pre-SRD with the control group having 74% (14 of the 19) of participants with *P. intermedia* at these levels, while the test group recorded 63% (12 of the 19). Twenty six percent (5 of 19) of the participants in the control group has *P. intermedia* above threshold levels, whereas 26% (7 of 19) of the participants in the test group had *P. intermedia* above detectable levels (Table 4.10).

No changes in prevalence of *P. intermedia* was observed in the control group post-SRD. In the test group, 13 of the 19 participants (68%) had *P. intermedia* below

detectable levels whereas 6 of the 19(32%) have *P. intermedia* at above detectable levels.

At twelve months, prevalence of *P. intermedia* at levels below threshold increased in both groups with the control group now having 16 of the 19 participants (84%) with these levels and the test group 15 of the 19 participants (79%). Only a few participants in both groups had *P. intermedia* above threshold levels (3 of 19 in the control group and 4 of 19 in the test group) (Table 4.10).

Table 4. 10 Prevalence of *P. intermedia* at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>P. intermedia</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	14(74%)	5(26%)	n=19 (100%)	12(63%)	7(37%)	n=19 (100%)
Three Months	14(74%)	5(26%)	n=19 (100%)	13(68%)	6(32%)	n=19 (100%)
Twelve Months	16(84%)	3(16%)	n=19 (100%)	15(79%)	4(21%)	n=19 (100%)

4.2.5.2 Analysis of Percentage of *P. intermedia* at each level pre-SRD, post-SRD and at twelve months

P. intermedia had the following changes in percentages for each level pre and post SRD and at twelve months (Table 4.11) (Figure 4.5):

- The percentage of *P. intermedia* at Level 0 or “bacterial load under cut off” (*P. intermedia* =<10₄), increased in the test group post-SRD (68.42%) and at twelve months (78.95%). No changes were observed in percentage of *P. intermedia* for

level 0 from baseline to three months (73.68%) in the control group, however an increase in percentage (84.21%) was observed at twelve months. (Figure 5.5.).

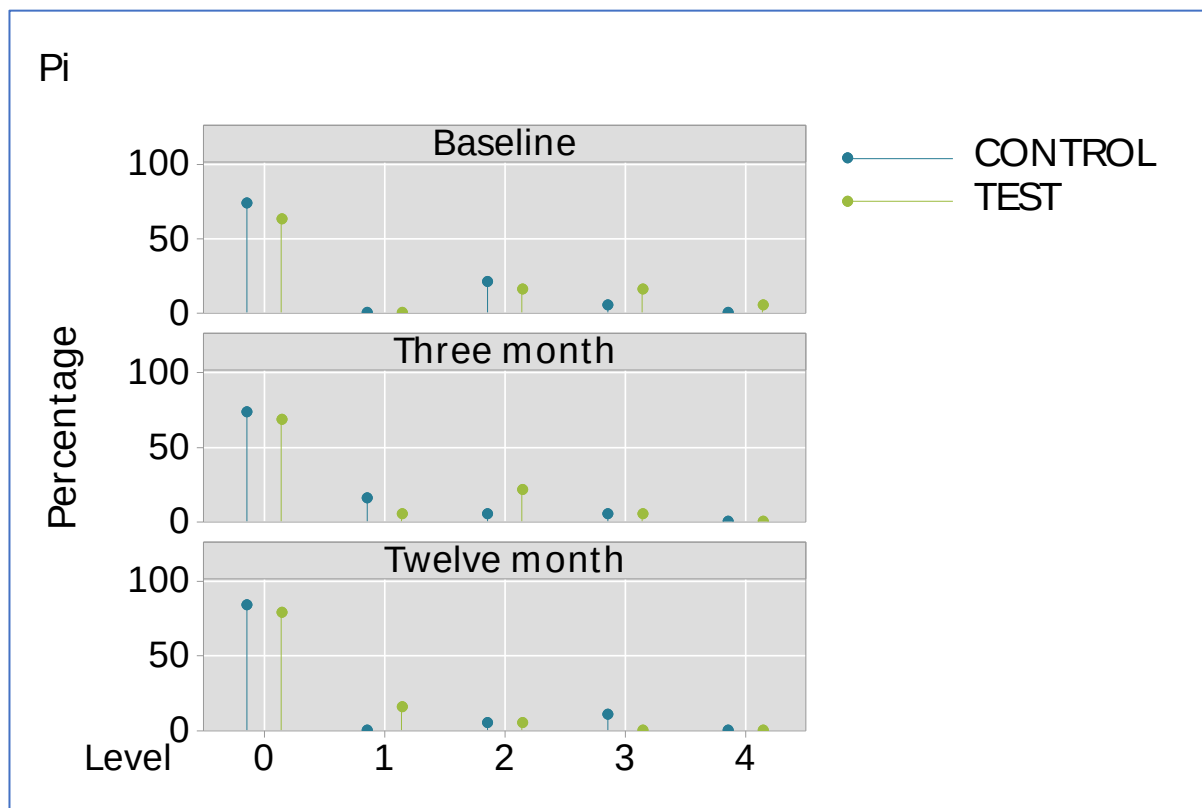
- For level 1 or “*Bacterial load at cut off*” (*P. intermedia* = 10^4), the percentage of *P. intermedia* at this level increased from 0% to 15.79% post-SRD and then decreased to 0% at twelve months in the control group. A similar observation was seen in the test group.
- The percentage of *P. intermedia* at level 2 or “*Increased Bacterial load*” (*P. intermedia* = $<10^5$) in the control group, decreased from 21.05% at pre-SRD to 5.26% post-SRD and remained at this percentage at twelve months. For the test group, the percentage of *P. intermedia* at level 2 increased from 15.79% pre-SRD to 21.05% post-SRD and then a decrease was noted at twelve months (5.26%).
- In the control group, for level 3 or “*High bacterial load*” (*P. intermedia* = $<10^6$), *P. intermedia* remained at 5.26% post -SRD and then increased to 10.53% at twelve months. In the test group, a decrease in percentage of *P. intermedia* for level 3 was noted post-SRD (5.26%), but this increased to 15.79% at twelve months.
- No observations for level 4 or “*Extremely high bacterial load*” (*P. intermedia* = $>10^6$) was seen in the control group. The test group recorded a 5.26% for level 4 of *P. intermedia* pre-SRD and this was reduced to 0% post-SRD and twelve months.

Table 4. 11 Percentage of *P. intermedia* at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	Rank/ Level	Percentage (Pre-SRD)	Percentage (Post-SRD)	↑/↓/0*	Percentage Twelve months	↑/↓/0*
<i>P. intermedia</i>						
Control	0	73.68	73.68	0	84.21	↑
Test	0	63.16	68.42	↑	78.95	↑
Control	1	0.00	15.79	↑	0.00	↓
Test	1	0.0	5.26	↑	0.00	↓
Control	2	21.05	5.26	↓	5.26	0
Test	2	15.79	21.05	↑	5.26	↓
Control	3	5.26	5.26	0	10.53	↑
Test	3	15.79	5.26	↓	15.79	↑
Control	4	0.00	0.00	0	0.00	0
Test	4	5.26	0.00	↓	0.00	0

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 5 A comparison of percentages for *P. intermedia* at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.2.6 *P. micros*

4.2.6.1 Prevalence of *P. micros*

All participants (100%) in the test and control group had *P. micros* above threshold levels pre-SRD. No changes in prevalence was noted in the control group post-SRD with all 19 participants still having *P. micros* above threshold levels. In the test group, there was an increase in prevalence of *P. micros* below detectable levels to 3 of 19 (16%) and 16 of 19 (84%) above threshold levels (Table 4.12).

The control group at twelve months had 1 of 19 (5%) of participants below threshold levels for *P. micros* and 18 of 19 (95%) above threshold levels. In the test group, only

2 of the 19 (11%) had *P. micros* at levels below threshold while 17 of 19 (89%) had levels above threshold for *P. micros* (Table 4.12).

Table 4. 12 Prevalence of *P. micros* at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>P. micros</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	0(0%)	19(100%)	n=19 (100%)	0(0%)	19(100%)	n=19 (100%)
Three Months	0(0%)	19(100%)	n=19 (100%)	3(16%)	16(84%)	n=19 (100%)
Twelve Months	1(5%)	18(95%)	n=19 (100%)	2(11%)	17(89%)	n=19 (100%)

4.2.6.2 Analysis of Percentage of *P. micros* at each level pre-SRD, post-SRD and at twelve months

The following changes were noted for *P. micros* pre, post SRD and at twelve months in test and control groups (Table 4.13) and (Figure 4.6):

- The percentage of *P. micros* for level 0 or “bacterial load under cut off” (*P. micros* = <10₄), was 0% pre and post SRD in the control group. The percentage increased to 5.26% at twelve months. In the test group, the percentage of *P. micros* at level 0, increased from 0% pre-SRD to 15.79% post-SRD and decreased to 10.53% at twelve months.
- For level 1 or “Bacterial load at cut off” (*P. micros* = 10₄), *P. micros* showed different trends in both groups. In the control group, there was a decrease to 0% post-SRD and then an increase to 15.79% at twelve months. The percentage of

P. micros for level 1 in the test group, increased to 5.26% post-SRD and then increased even further to 26.32% at twelve months (Figure 5.6.).

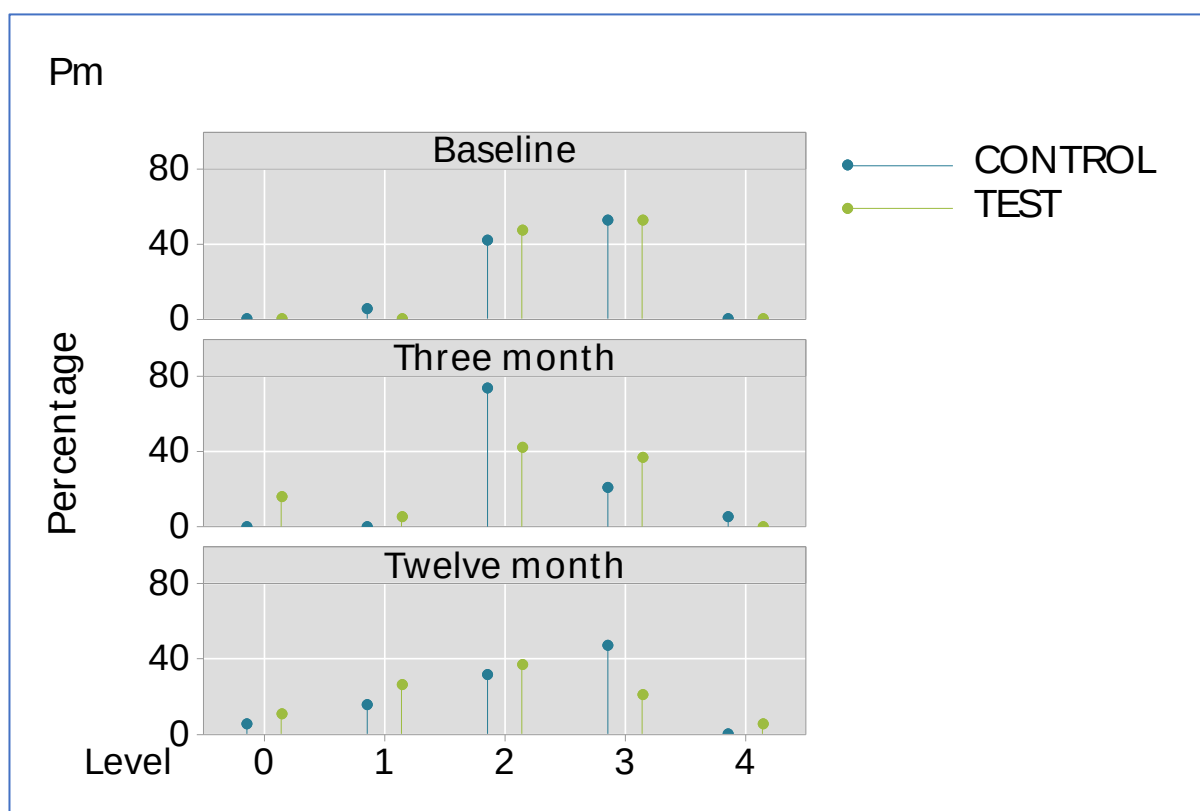
- The percentage of *P. micros* at level 2 or “*increased Bacterial load*” (*P. micros* = <10⁵), increased from 42.11% pre-SRD to 73.69% post-SRD and then decreased to 31.58% at twelve months in the control group. In the test group, the percentage of *P. micros* at level 2, decreased to 42.11% post-SRD and then decreased further to 36.84% at twelve months.
- In the control group, the percentage of *P. micros* at Level 3 or “*High bacterial load*” (*P. micros* = <10⁶), decreased post-SRD to 21.05% and then increased to 47.37% at twelve months. In the test group, the percentage of *P. micros* at level 3 decreased post-SRD and at twelve months.
- For level 4 or “*Extremely high bacterial load*” (*P. micros* = >10⁶), there were no evidence of *P. micros* at this level at any time point for the test group. In the control group, a slight increase was noted post-SRD (from 0% to 5.26%); however, this was reduced to 0% at twelve months

Table 4. 13 Percentage of *P. micros* at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	*Rank	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage twelve months	↑/↓/0*
<i>P. micros</i>						
Control	0	0.00	0.00	0	5.26	↑
Test	0	0.00	15.79	↑	10.53	↓
Control	1	5.26	0.00	↓	15.79	↑
Test	1	0.00	5.26	↑	26.32	↑
Control	2	42.11	73.68	↑	31.58	↓
Test	2	47.37	42.11	↓	36.84	↓
Control	3	52.63	21.05	↓	47.37	↑
Test	3	52.63	36.84	↓	21.05	↓
Control	4	0.00	5.26	↑	0.00	↓
Test	4	0.00	0.00	0	0.00	0

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 6 A comparison of percentages for *P. micros* at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.2.7 *F. nucleatum*

4.2.7.1 Prevalence of *F. nucleatum*

All participants in the control group (100%) had *F. nucleatum* was above threshold levels pre-SRD. In the test group, only 1 of the 19 participants (5%) had *F. nucleatum* below threshold levels with 18 of the 19 (95%) having levels above threshold (Table 4.14).

Post-SRD saw no changes in prevalence of *F. nucleatum* below and above threshold for both groups. At twelve months, all participants in both the test and control group recorded *F. nucleatum* at above threshold levels (Table 4.14).

Table 4. 14 Prevalence of *F. nucleatum* at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>F. nucleatum</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	0(0%)	19(100%)	n=19 (100%)	1(5%)	18(95%)	n=19 (100%)
Three Months	0(0%)	19(100%)	n=19 (100%)	1(5%)	18(95%)	n=19 (100%)
Twelve Months	0(0%)	19(100%)	n=19 (100%)	0(0%)	19(100%)	n=19 (100%)

4.2.7.2 Analysis of Percentage of *F. nucleatum* at each level pre-SRD, post-SRD and at twelve months

The following changes in percentages were observed for *F. nucleatum* (Table 4.15) (Figure 4.7)

- The percentage of *F. nucleatum* at level 0 or “*bacterial load under cut off*” (*Fusobacterium nucleatum*=<10₄), remained at 0% in the control group at all three time intervals. In the test group, no changes were observed in percentage of level 0 (5.26%) for *F. nucleatum* pre- and post-SRD, however at twelve months there was a decrease to 0%.
- For *F. nucleatum* at level 1 or “*Bacterial load at cut off*” (*F. nucleatum* =10₄), the percentage for *Fusobacterium nucleatum*, remained at 5.26% in the control group post -SRD and decreased to 0% at twelve months. The percentage of *F. nucleatum* at level 1 in the test group 1 was recorded at 0% pre and post-SRD and then increased to 5.26% at twelve months.

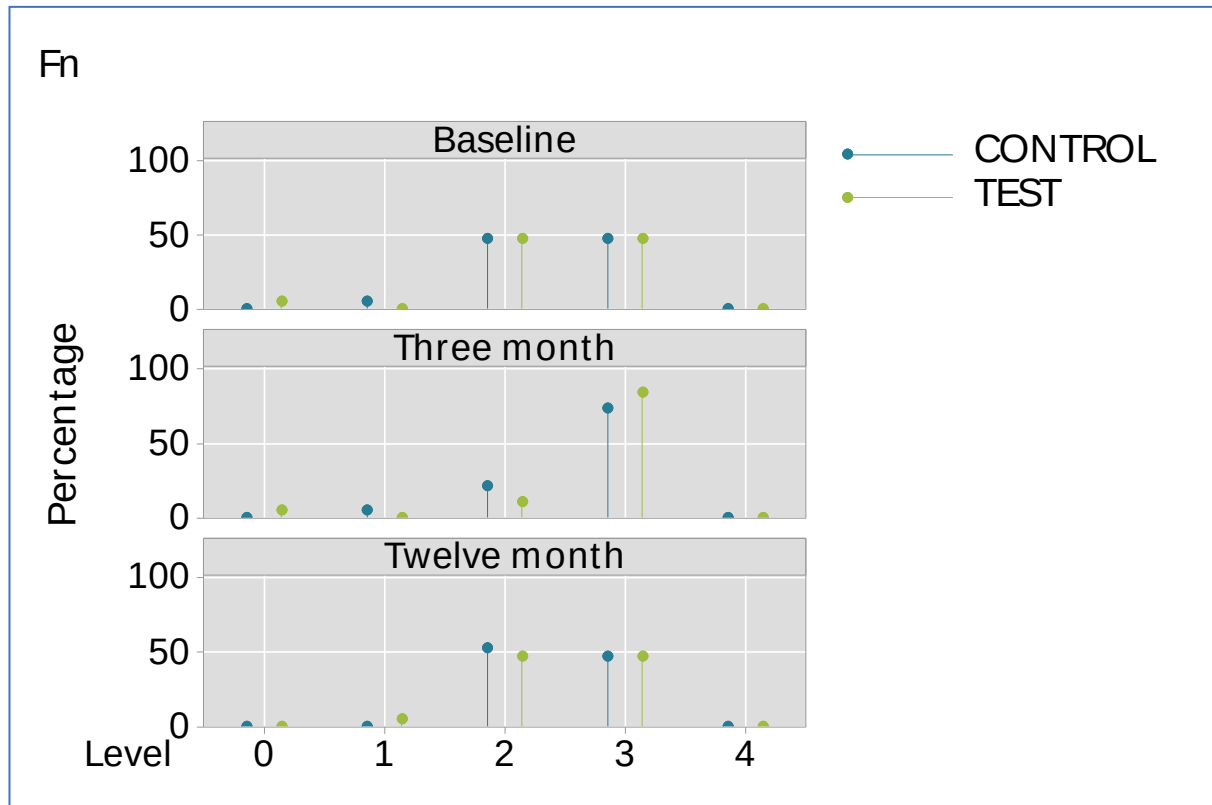
- The percentages recorded for *F. nucleatum* at Level 2 or “+Increased Bacterial load” (*F. nucleatum* = $<10^5$) decreased in both groups post-SRD; however, increased at twelve months with the test group having a higher percentage of *F. nucleatum* at level 2 at twelve months (47.37%).
- For Level 3 or “++ High bacterial load” (*F. nucleatum* = $<10^6$), both the test and control groups had increases in percentages of *F. nucleatum* post -SRD and then decreased at twelve months to pre-SRD percentages recorded (47.37%).
- There were no observations of *F. nucleatum* at Level 4 or “+++ Extremely high bacterial load” (*F. nucleatum* = $>10^6$) for both groups pre, post-SRD and at twelve months.

Table 4. 15 Percentage of *F. nucleatum* at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	*Rank	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage twelve months	↑/↓/0*
<i>F. nucleatum</i>						
Control	0	0.0	0.00	0	0.00	0
Test	0	5.26	5.26	0	0.00	↓
Control	1	5.26	5.26	0	0.00	↓
Test	1	0.00	0.00	0	5.26	↑
Control	2	47.37	21.05	↓	26.32	↑
Test	2	47.37	10.53	↓	47.37	↑
Control	3	47.37	73.68	↑	47.37	↓
Test	3	47.37	84.21	↑	47.37	↓
Control	4	0.0	0.00	0	0.00	0
Test	4	0.0	0.00	0	0.00	0

- ↑ - increase in percentage of rank
- ↓ - decrease in percentage of rank
- 0 - no changes in percentage of rank.

Figure 4. 7 A comparison of percentages for *F. nucleatum* at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.2.8 *C. rectus*

4.2.8.1 Prevalence of *C. rectus*

C. rectus was found above threshold levels in all patients in the control group pre-SRD and in 84% of the patients in the test group. Sixteen percent of participants in the test group had *C. rectus* below threshold levels. Changes in prevalence of *C. rectus* occurred in the control group post-SRD, with 21% of participants now having *C. rectus* at levels below threshold and 84% with *C. rectus* above threshold levels. No changes in prevalence were observed in the test group. At twelve months, prevalence of *C. rectus* in the control group improved further for level below threshold with 26% of participants having *C. rectus* at this level and only 74% having *C. rectus* above

threshold levels. Improvement in prevalence were also noted for the test group at twelve months, with 37% of participants having *C. rectus* below threshold and 63% above threshold levels (Table 4.16).

Table 4. 16 Prevalence of *C. rectus* at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>C. rectus</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	0(0%)	19(100%)	n=19 (100%)	3(16%)	16(84%)	n=19 (100%)
Three Months	4(21%)	15(79%)	n=19 (100%)	3(16%)	16(84%)	n=19 (100%)
Twelve Months	5(26%)	14(74%)	n=19 (100%)	7(37%)	12(63%)	n=19 (100%)

4.2.8.2 Analysis of Percentage of *C. rectus* at each level pre-SRD, post-SRD and at twelve months

The following changes in percentages were recorded for *C. rectus* at each level (Table 4.17). Figure 4.8 displays the percentage of *C. rectus* at each level and at each time point.

- The percentage of *C. rectus* at level 0 or “bacterial load under cut off” (*C. rectus* ≤ 10₄) was 0% at baseline in the control group and increased at both time intervals i.e. three and twelve months. In the test group, the percentage of *C. rectus* at level 0, at baseline was 15.79% and remained at 15.79% at three

months. At twelve months a higher percentage of *C. rectus* was recorded (36.84%) at level 0 in the test group.

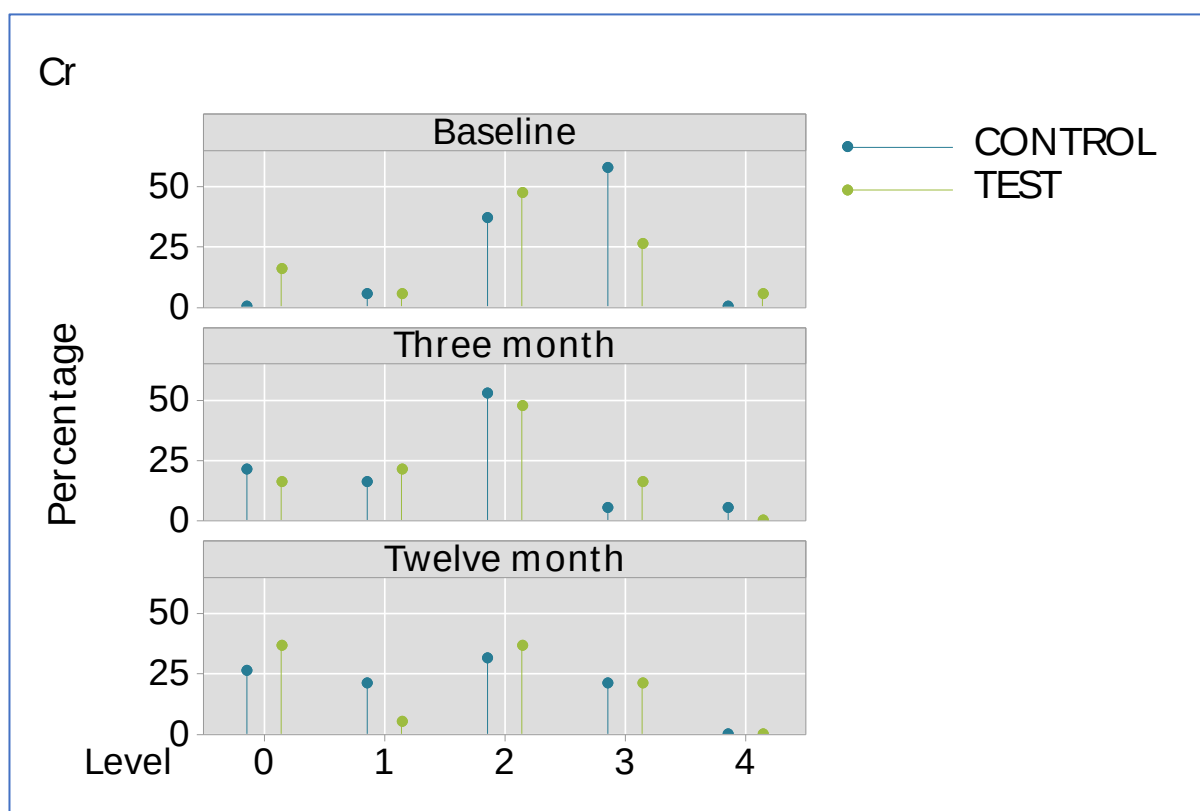
- There were increases in percentage of *C. rectus* for level 1 or “*Bacterial load at cut off*” (*C. rectus*= 10^4) in the control group, from baseline to three months and again at twelve months. In the test group, the percentage of *C. rectus* at level 1 increased at three months (21.05%) and returned to the baseline percentage of 5.26% at twelve months.
- The percentages recorded for *C. rectus* at Level 2 or “*Increased Bacterial load*” (*C. rectus* = $<10^5$) in the control group, increased at three months from 36.84% at baseline to 52.63% at three months; however, the percentage decreased to 31.58% at twelve months. The test group recorded the same percentage (47.37%) at baseline and post-SRD and decreased to 21.05% at twelve months.
- A substantial decrease was noted in the control group for Level 3 or “*High bacterial load*” (*C. rectus* = $<10^6$) from 57.89% at baseline to 5.26% at three months. At twelve months an increase in percentage of *C. rectus* was recorded (21.05%). *C. rectus* decreased from baseline to three months in the test group for level 3 but an increase in percentage (21.05%) at twelve months was noted.
- Percentage of *C. rectus* at level 4 or “*Extremely high bacterial load*” (*C. rectus* = $>10^6$) was 0% at baseline, increased to 5.26% at three months and then decreased to 0% at twelve months in the control group. In the test group, the percentage of *C. rectus* at level 4, decreased from 5.26% to 0% at three months and this percentage remained at 0% at twelve months.

Table 4. 17 Percentage of *C. rectus* at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	Rank/ Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage twelve months	↑/↓/0*
<i>C. rectus</i>						
Control	0	0.0	21.05	↑	26.32	↑
Test	0	15.79	15.79	0	36.84	↑
Control	1	5.26	15.79	↑	21.05	↑
Test	1	5.26	21.05	↑	5.26	↓
Control	2	36.84	52.63	↑	31.58	↓
Test	2	47.37	47.37	0	36.84	↓
Control	3	57.89	5.26	↓	21.05	↑
Test	3	26.32	15.79	↓	21.05	↑
Control	4	0.0	5.26	↑	0.00	↓
Test	4	5.26	0.00	↓	0.00	0

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 8 A comparison of percentages for *C. rectus* at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.2.9 *E. nodatum*

4.2.9.1 Prevalence of *E. nodatum*

The prevalence of *E. nodatum* below threshold was 5 of 19(26%) in the control group and 4 of 19(21%) in the test group. Fourteen of nineteen (74%) of participants had *E. nodatum* above threshold levels with 15 of 19(79%) participants at this level pre-SRD (Table 4.18).

Post-SRD saw increases in prevalence of participants in both groups for *E. nodatum* below detectable levels. Eight of the nineteen participants in the control group had levels below threshold and 10 of 19 participants in the test group recorded *E. nodatum* at below detectable levels. Fifty eight percent (11 of 19) of participants had *E. nodatum* above threshold levels in the control group with 9 of 19 (47%) recording *E. nodatum*

above threshold post-SRD. At twelve months, the prevalence of *E. nodatum* for below threshold levels increased to 11 of 19 (47%) participants in the control group, while the test group has 9 of 19 participants with levels below threshold. For levels above threshold the control group had 8 of 19 (42%) participants, with the test group having 10 of 19 (53%) at these levels (Table 4.18).

Table 4. 18 Prevalence of *E. nodatum* at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>E. nodatum</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	5(26%)	14(74%)	n=19 (100%)	4(21%)	15(79%)	n=19 (100%)
Three Months	8(42%)	11(58%)	n=19 (100%)	10(53%)	9(47%)	n=19 (100%)
Twelve Months	11(58%)	8(42%)	n=19 (100%)	9(47%)	10(53%)	n=19 (100%)

4.2.9.2. Analysis of Percentage of *E. nodatum* at each level pre-SRD, post-SRD and at twelve months

The following changes were seen for *E. nodatum* at each level (Table 4.19). Figure 4.9 displays percentages of *E. nodatum* at each level pre, post-SRD and at twelve months for both treatment groups:

- There were increases in percentage of *E. nodatum* at level 0 or “bacterial load under cut off” (*E. nodatum* =<10₄) at three and twelve months in the control group; however, in the test group, there was an increase in percentage of *E.*

nodatum at level 0 post-SRD (52.63%) and then a decrease to 47.37% at twelve months.

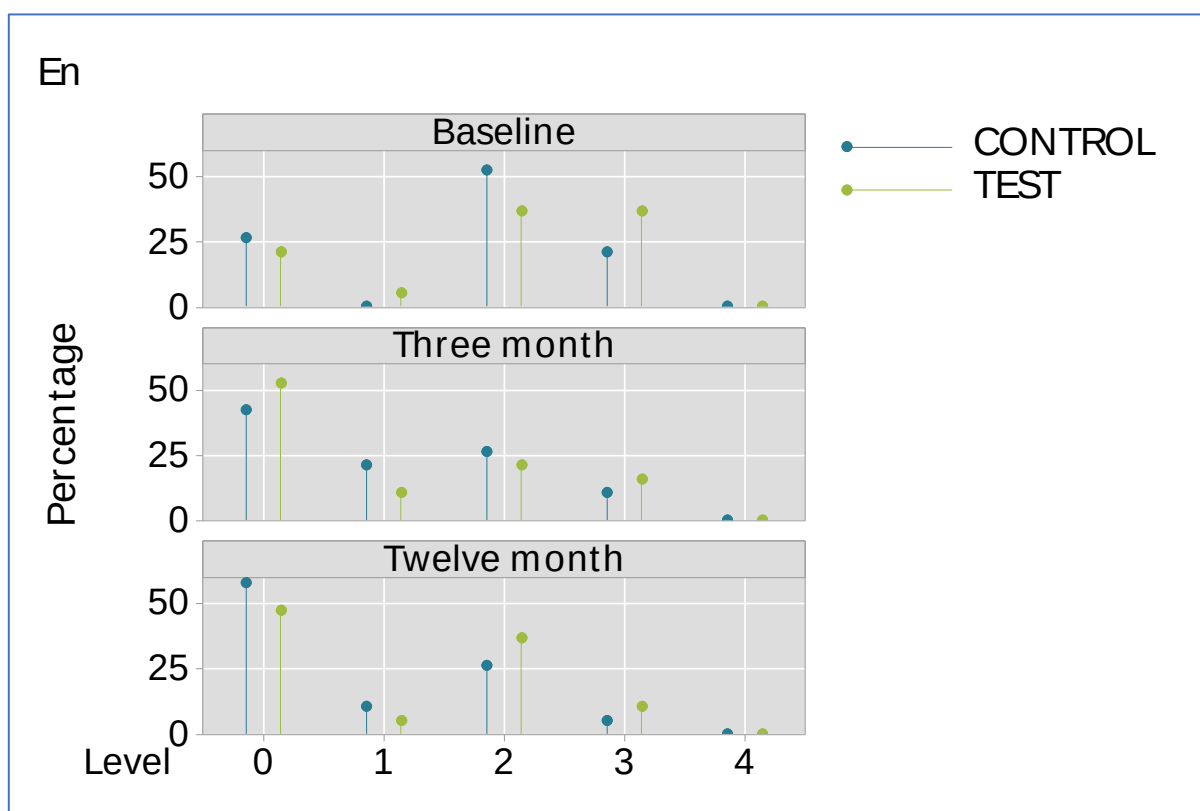
- For bacterial load at cut off level 1 or “*Bacterial load at cut off*” (*E. nodatum* = 10^4) for *E. nodatum*, both the test and control groups displayed similar patterns, where there were increases in percentages of *E. nodatum* at this level at three months and then decrease in percentage at twelve months.
- Both the test and control groups showed decreases in percentages for Level 2 or “*Increased Bacterial load*” (*E. nodatum* = $<10^5$) at three and twelve months.
- A decrease in percentage for *E. nodatum* at level 3 or “*High bacterial load*” (*E. nodatum* = $<10^6$) for both test and control groups were observed post-SRD and at twelve months.
- There were no recorded levels of level 4 or “*Extremely high bacterial load*” (*E. nodatum* = $>10^6$) of *E. nodatum* for both groups.

Table 4. 19 Percentage of *E. nodatum* at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	Rank/ Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage twelve months	↑/↓/0*
<i>E. nodatum</i>						
Control	0	26.32	42.11	↑	57.89	↑
Test	0	21.05	52.63	↑	47.37	↓
Control	1	0.0	21.05	↑	10.53	↓
Test	1	5.26	10.53	↑	5.26	↓
Control	2	52.63	26.32	↓	26.32	0
Test	2	36.84	15.79	↓	10.53	↓
Control	3	21.05	10.53	↓	5.26	↓
Test	3	36.84	15.79	↓	10.53	↓
Control	4	0.0	0.00	0	0.00	0
Test	4	0.0	0.00	0	0.00	0

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 9 A comparison of percentages for *E. nodatum* at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.2.10 *E. corrodens*

4.2.10.1 Prevalence of *E. corrodens*

All participants (100%) had detectable levels of *E. corrodens* pre-SRD in the control group. In the test group 2 of 19 (11%) had levels below threshold and 17 of 19 (89%) has levels above threshold (Table 4.20).

Post-SRD, 2 of 19 (11%) of participants had levels below threshold and 17 of 19 (89%) had above threshold levels. In the test group, 1 of 19 (5%) participants had below threshold levels and 18 of 19 (95%) participants had levels above threshold.

At twelve months, prevalence of *E. corrodens* below threshold increased in both groups to 4 of 19 (21%) in the control group and 3 of 19 (16%) in the test group (Table 4.20).

Table 4. 20 Prevalence of *E. corrodens* at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>E. corrodens</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	0(0%)	19(100%)	n=19 (100%)	2(11%)	17(89%)	n=19 (100%)
Three Months	2(11%)	17(89%)	n=19 (100%)	1(5%)	18(95%)	n=19 (100%)
Twelve Months	4(21%)	15(79%)	n=19 (100%)	3(16%)	16(84%)	n=19 (100%)

4.2.10.2. Analysis of Percentage of *E. corrodens* at each level pre-SRD, post-SRD and at twelve months

The following changes were noted for *E. corrodens* (Table 4.21) (Figure 4.10)

- The percentage of *E. corrodens* at level 0 or “bacterial load under cut off” (*E. corrodens*=<10₄), increased at three months and twelve months in the control group. In the test group, there was a decrease in percentage of *E. corrodens* at level 0 at three months and then an increase at twelve months.
- For level 1 or “Bacterial load at cut off” (*E. corrodens* =10₄), the percentage of *E. corrodens* increased at three and twelve months in the control group,

whereas in the test group, the percentage of *E. corrodens* at level 1 remained the same as baseline at three months and then increased at twelve months.

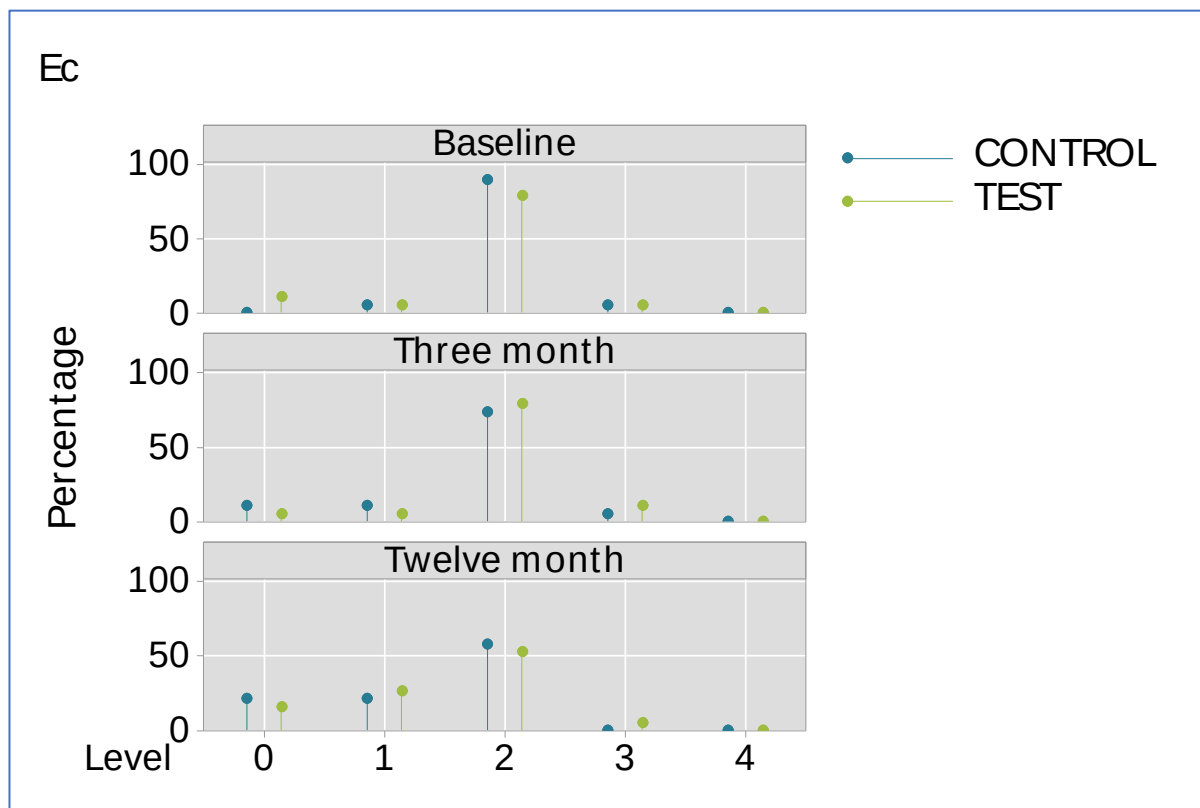
- *E. corrodens* at level 2 or “*Increased Bacterial load*” (*E. corrodens* = $<10^5$), had a decrease in percentage in the control group from baseline to three months and decreased even further at twelve months. In the test group, the percentage of *E. corrodens* at level 2, remained at 78.95% at baseline and three months and decreased to 52.63% at twelve months.
- The percentage of *E. corrodens* at level 3 or “*High bacterial load*” (*E. corrodens* = $<10^6$) was unchanged at pre and post-SRD in the control group and then decreased to 0% at twelve months. In the test group, the percentage of *E. corrodens* at level 3 increased at three months to 10.53% and then returned to the baseline percentage of 5.26% at twelve months.
- There was 0% of level 4 or “*Extremely high bacterial load*” (*E. corrodens* = $>10^6$) of *E. corrodens* in both test and control groups for each time point.

Table 4. 21 Percentage of *E. corrodens* at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	Rank/ Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage twelve months	↑/↓/0*
<i>E. corrodens</i>						
Control	0	0.0	10.53	↑	21.05	↑
Test	0	10.53	5.26	↓	15.79	↑
Control	1	5.26	10.53	↑	21.05	↑
Test	1	5.26	5.26	0	26.32	↑
Control	2	89.47	73.68	↓	57.89	↓
Test	2	78.95	78.95	0	52.63	↓
Control	3	5.26	5.26	0	0.00	↓
Test	3	5.26	10.53	↑	5.26	↓
Control	4	0.0	0.00	0	0.00	0
Test	4	0.0	0.00	0	0.00	0

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 10 A comparison of percentages for *E. corrodens* at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.2.11 *Capnocytophaga* sp. (*sputigena*; *gingivalis*; *ochracea*)

4.2.11.1 Prevalence of *Capnocytophaga* sp.

No changes in prevalence of *Capnocytophaga* sp. below threshold and above threshold were observed in the test and control groups pre-SRD, post-SRD and at twelve months (Table 4.22).

In the control group, the prevalence of *Capnocytophaga* sp. remained at 3 of 19 (16%) for levels below threshold and 16 of 18 (84%) for levels above threshold throughout the study period. In the test group, 1 of 19 (5%) of participants had levels below threshold and 18 of 19 (95%) had levels above threshold with no changes observed at each time point (Table 4.22).

Table 4. 22 Prevalence of *Capnocytophaga sp.* at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups

	Control (n=19)			Test (n=19)		
<i>Capnocytophaga sp.</i>						
Time Point	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total	Below Threshold Aa <10 ₃	Above Threshold Aa >10 ₃	Total
Baseline	3(16%)	16(84%)	n=19 (100%)	1(5%)	18(95%)	n=19 (100%)
Three Months	3(16%)	16(84%)	n=19 (100%)	1(5%)	18(95%)	n=19 (100%)
Twelve Months	3(16%)	16(84%)	n=19 (100%)	1(5%)	18(95%)	n=19 (100%)

4.2.11.2 Analysis of Percentage of *Capnocytophaga sp.* at each level pre-SRD, post-SRD and at twelve months

Table 4.23 and Figure 4.11 shows changes in percentages of *Capnocytophaga sp.* at each level at each time point. The following changes were observed:

- The percentage of *Capnocytophaga sp.* at level 0 or “*bacterial load under cut off*” (*Capnocytophaga sp.* = <10₄), remained the same for control and test group at all three time points.
- For level 1 or “*Bacterial load at cut off*” (*Capnocytophaga sp.* = 10₄), similar patterns were seen in the test and control group in terms of increases in percentage post-SRD and then a decrease in percentage of *Capnocytophaga sp.* at twelve months and percentages returning to baseline percentages.
- For *Capnocytophaga sp.* at level 2 or “*Increased Bacterial load*” (*Capnocytophaga sp.* = <10₅) in the control group, the percentage decreased

from baseline to post-SRD and then increased at twelve months. In the test group, increases in percentages were seen post-SRD and twelve months.

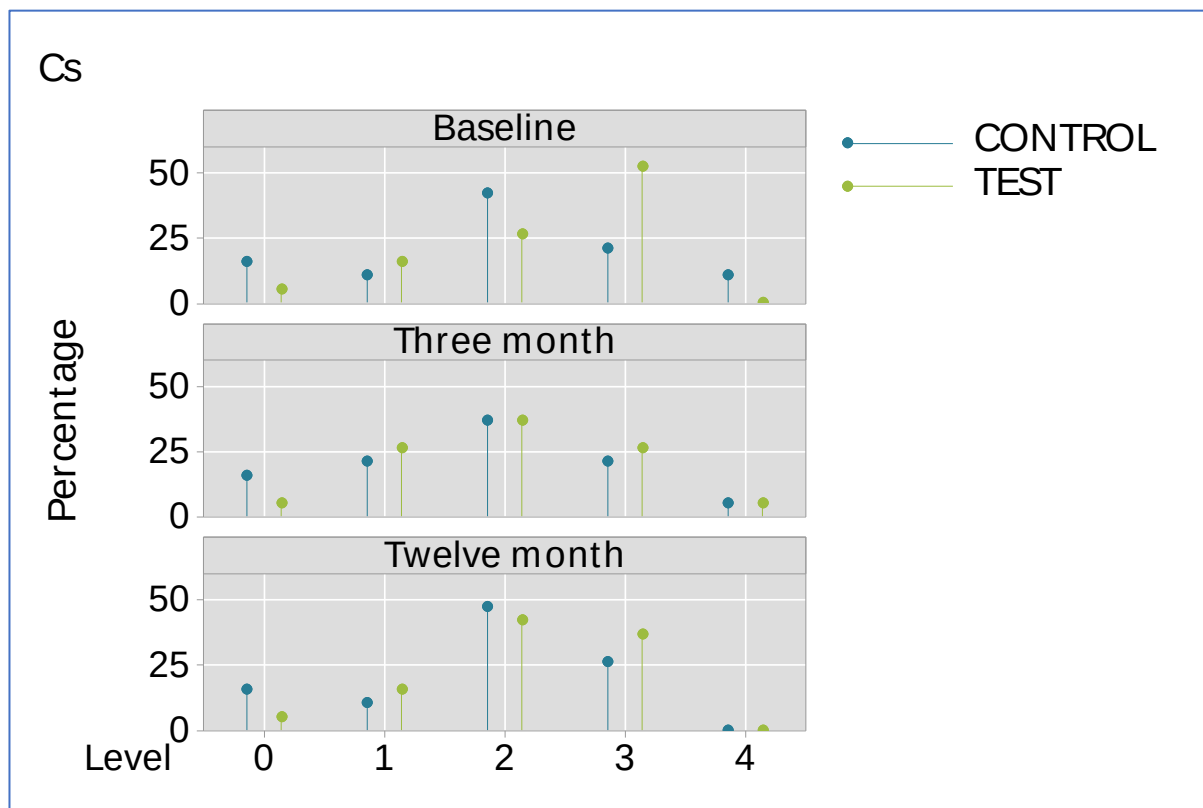
- The percentages of *Capnocytophaga sp.* at level 3 or “*High bacterial load*” (*Capnocytophaga sp.* = <10⁶), remained at the same percentage at baseline and post-SRD in the control group and then increased at twelve months. In the test group, there was a decrease in percentage of *Capnocytophaga sp.* at level 3 post-SRD, but at twelve months, an increase in percentage was noted.
- For level 4 or “*Extremely high bacterial load*” (*Capnocytophaga sp.* = >10⁶), the percentage of *Capnocytophaga sp.* decreased at three months in the control group and decreased even further and was recorded at 0% at twelve months. In the test group, there was an increase in percentage of *Capnocytophaga sp.* at level 4 post-SRD, and then a decrease to 0% at twelve months.

Table 4. 23 Percentage of *Capnocytophaga sp.* at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	Rank/ Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage twelve months	↑/↓/0*
<i>Capnocytophaga sp.</i>						
Control	0	15.79	15.79	0	15.79	0
Test	0	5.26	5.26	0	5.26	0
Control	1	10.53	21.05	↑	10.53	↓
Test	1	15.79	26.32	↑	15.79	↓
Control	2	42.11	36.84	↓	47.37	↑
Test	2	26.32	36.84	↑	42.11	↑
Control	3	21.05	21.05	0	26.32	↑
Test	3	52.63	26.32	↓	36.84	↑
Control	4	10.53	5.26	↓	0.00	↓
Test	4	0.0	5.26	↑	0.00	↓

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 11 A comparison of percentages for *Capnocytophaga* sp. at each level at baseline (pre-SRD), three (post-SRD) and twelve months for control and test groups



4.3 Analyses of the bacterial complexes

Table 4.24 provides baseline, three and twelve-month statistical data obtained from the Mann Whitney *U* tests (mean rank, *U*, z-value and p-value) for the complexes of bacteria; namely red, orange, orange-associated complex and green complex.

Table 4. 24 Mean Rank, *U*, z-value and p-value for the complexes of bacteria at baseline, three and twelve months for control and test groups

	Control n=19	Test n=19	Test Statistics		
Pathogen	Mean Rank	Mean Rank	<i>U</i>	z-value	p- value*
Red complex					
Baseline	20.21	18.79	167.00	-0.448	0.75
Three months	17.92	21.08	150.50	-0.985	0.37
Twelve months	21.53	17.47	142.00	-1.231	0.23
Orange complex					
Baseline	18.24	20.76	156.50	-0.879	0.55
Three months	19.29	19.71	176.50	-0.139	0.99
Twelve months	21.03	17.97	151.50	-0.959	0.41
Orange – associated complex					
Baseline	19.74	19.26	176.00	-0.139	0.91
Three months	19.87	19.13	173.50	-0.213	0.85
Twelve months	18.53	20.47	162.00	-0.569	0.60
Green complex					
Baseline	18.71	20.29	165.50	-0.470	0.69
Three months	18.24	20.27	156.50	-0.738	0.49
Twelve months	18.42	20.58	160.00	-0.633	0.60

*N<20, exact significance reported

4.3.1 Baseline (Pre-SRD)

At baseline, the mean rank of the red and orange associated complex appeared more frequently in higher ranked positions in the control group as compared to the test group. This is suggestive that the red and orange associated complex bacteria were found in higher numbers in the control group than the test group. The orange and green complex had higher numbers in the test group as compared to the control group pre-SRD. However, no statistical significance was found for differences in mean ranks of bacterial complexes between groups at baseline ($p>0.05$)

4.3.2 Three Months (Post-SRD)

Table 4.24 provides baseline statistical data obtained from the Mann Whitney *U* tests for the complexes of bacteria. At three months, the red, orange and green complexes appeared to more frequently in higher ranked positions in the test group as compared to the control group, suggesting higher numbers of these complexes of bacteria in the test group. The orange associated complex had higher numbers of bacteria in the control group than the test group post-SRD. No statistical significance was reported between groups for mean ranks of bacterial complexes ($p>0.05$) at three months.

4.3.3 Twelve Months

Statistical analysis revealed changes in mean ranks at twelve months (Table 4.24). The mean rank of the red and orange complexes appeared in higher ranked positions or higher numbers in the control group at twelve months when compared to the test group, whereas the green and orange-associated complexes had higher numbers in the test group as compared to the control group. However, these results were not statistically significant ($p>0.05$).

4.3.4 Analysis percentages of the bacterial complexes at each level pre-SRD, post-SRD and twelve months

4.3.4.1 Red Complex (*P. gingivalis*, *T. forsythia*, *T. denticola*)

The following changes in percentages were noted for the treatment groups. (Table 4.25, Figure 4.12)

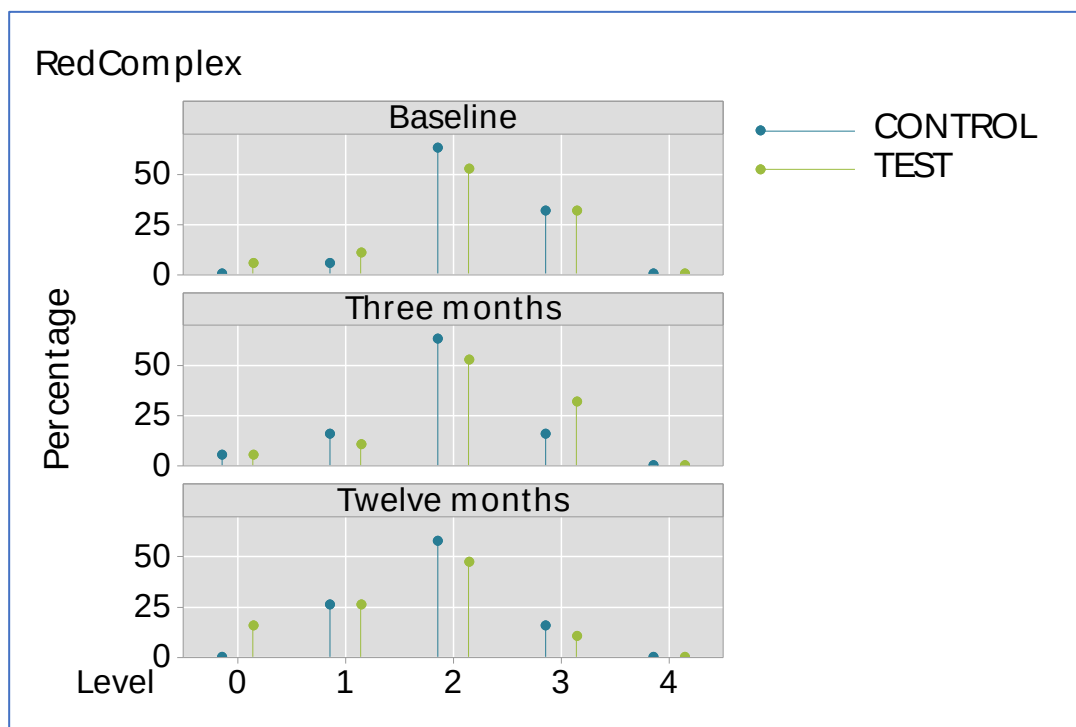
- For “*bacterial load below cut off*” or level 0, the percentage of the red complex bacteria increased post-SRD and then decreased to baseline levels at twelve months in the control group. In the test group, however there was no changes noted in percentage of the red complex at level 0 post-SRD, however at twelve months there was an increase in percentage of red complex for level 0 to 15.8%.
- The control group displayed increases in percentages for “*bacterial load at cut off*” or level 1 post-SRD and twelve months, whereas in the test group, no changes in percentages were recorded from baseline to post-SRD and then an increase in percentage of red complex bacteria at level 1 was seen at twelve months.
- For level 2 or “*Increased bacterial Load*”, the percentage of the red complex showed no changes in percentages pre and post-SRD for both groups. However, decreases in percentage of red complex bacteria at level 2 were recorded at twelve months in both groups.
- The control group had a decrease in percentages for level 3 or “*high bacterial load*” of the red complex from baseline to post-SRD and remained the same at twelve months. In the test group, no changes were observed post-SRD. However, further decreases in percentage of level 3 of the red complex was seen at twelve months.
- There were 0% of level 4 or “*extremely high bacterial load*” for both test and control groups for all three time intervals.

Table 4. 25 Percentage of the red complex at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	*Rank/ Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage Twelve Months	↑/↓/0*
Red complex						
Control	0	0.0	5.3	↑	0.0	↓
Test	0	5.3	5.3	0	15.8	↑
Control	1	5.3	15.8	↑	26.3	↑
Test	1	10.5	10.5	0	26.3	↑
Control	2	63.2	63.2	0	57.9	↓
Test	2	52.6	52.6	0	47.4	↓
Control	3	31.6	15.8	↓	15.8	0
Test	3	31.6	31.6	0	10.5	↓
Control	4	0.0	0.0	0	0.0	0
Test	4	0.0	0.0	0	0.0	0

- ↑ - increase in percentage of rank
- ↓ - decrease in percentage of rank
- 0 - no changes in percentage of rank.

Figure 4. 12 A comparison of percentages for the red complex at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups



4.3.4.2 Orange Complex (*P. intermedia*, *P. micro* and *F. nucleatum*)

The following changes were observed in the test and control groups. (Table 4.26)
Figure 4.13 displays the percentage of the orange complex for each level.

- For level 0 or “*bacterial load under cut off*” for the orange complex, there were 0% recorded for the control group at baseline, three and twelve months. In the test group, there was an increase in percentage from 0% at baseline to 5.3% at three months; however, at twelve months the percentage of orange complex under cut off returned to the baseline percentage of 0%.
- Both the test and control groups showed increases in percentage of level 1, “*bacterial load at cut off*” at three and twelve months.

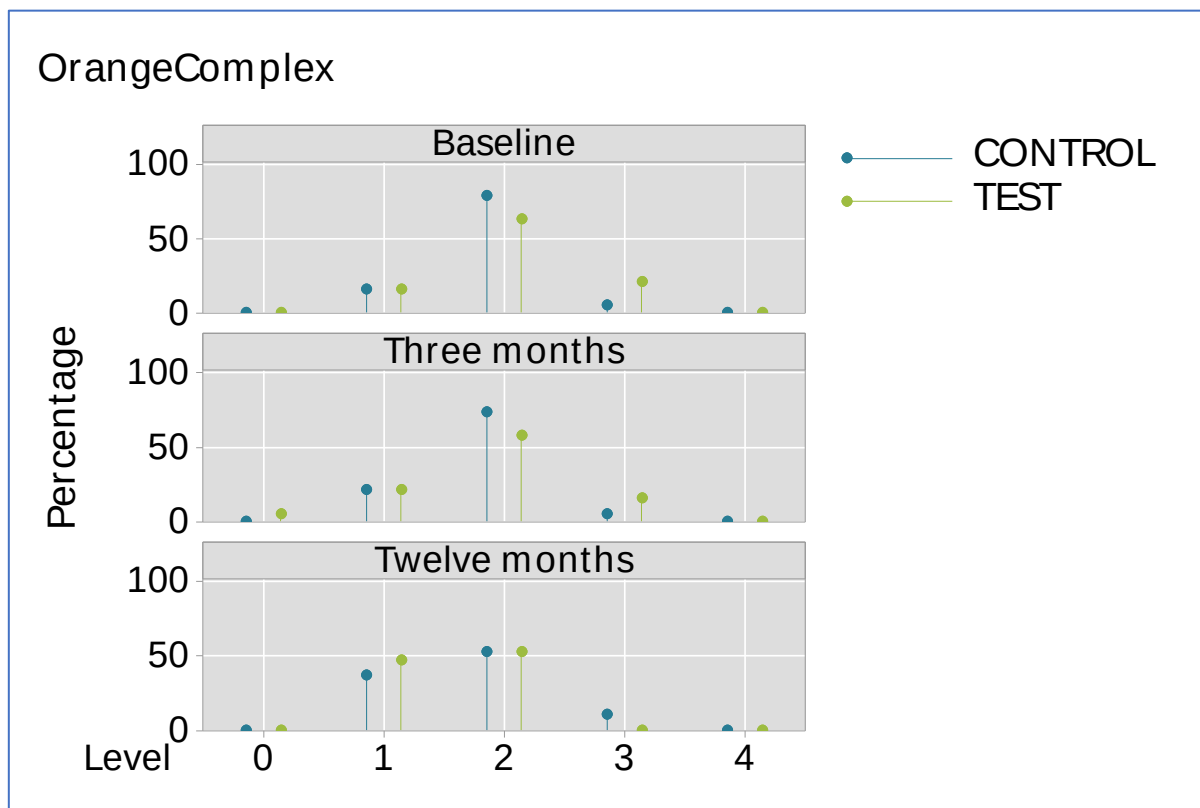
- For “*increased bacterial load*” or level 2, the orange complex, decreased in both test and control groups at three and twelve months.
- For level 3 or “*high bacterial loads*”, there was an increase in percentage on the control group at twelve months. In the test group, percentage of the orange complex at level 3, decreased at both time points, that is post-SRD (15.8%) and twelve months (0%)
- There was 0% of level 4 or “*extremely high bacterial load*” of the orange complex recorded at all three time points in both groups.

Table 4. 26 Percentage of the orange complex at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	Rank/ Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage Twelve Months	↑/↓/0*
ORANGE COMPLEX						
Control	0	0.0	0.0	0	0.0	0
Test	0	0.0	5.3	↑	0.0	↓
Control	1	15.8	21.1	↑	36.8	↑
Test	1	15.8	21.1	↑	47.4	↑
Control	2	78.9	73.7	↓	52.6	↓
Test	2	63.2	57.9	↓	52.6	↓
Control	3	5.3	5.3	0	10.5	↑
Test	3	21.1	15.8	↓	0.0	↓
Control	4	0.0	0.0	0	0.0	0
Test	4	0.0	0.0	0	0.0	0

- ↑ - increase in percentage of rank
- ↓ - decrease in percentage of rank
- 0 - no changes in percentage of rank.

Figure 4. 13 A comparison of percentages for the orange complex at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups



4.3.4.3 Orange- associated Complex (*C. rectus* and *E. nodatum*)

The following changes in percentages of levels were observed (Table 4.27, Figure 4.14)

- The control group showed an increase in percentage of the orange-associated complex for level 0 or “*bacterial load under cut off*” from 0% at baseline to 31.6% at three months. This was maintained at 31.6% at twelve months. In the test group, there was an increase in percentage of level 0 at three months, however this percentage decreased somewhat from 36.8% to 31.6% at twelve months.
- The percentage of the orange-associated complex at level 1 or “*bacterial level at cut off*”, decreased at three months in the control group and then increased

at twelve months. This was different for the test group, where there was an increase in percentage of orange-associated complex for level 1 at three months and at twelve months.

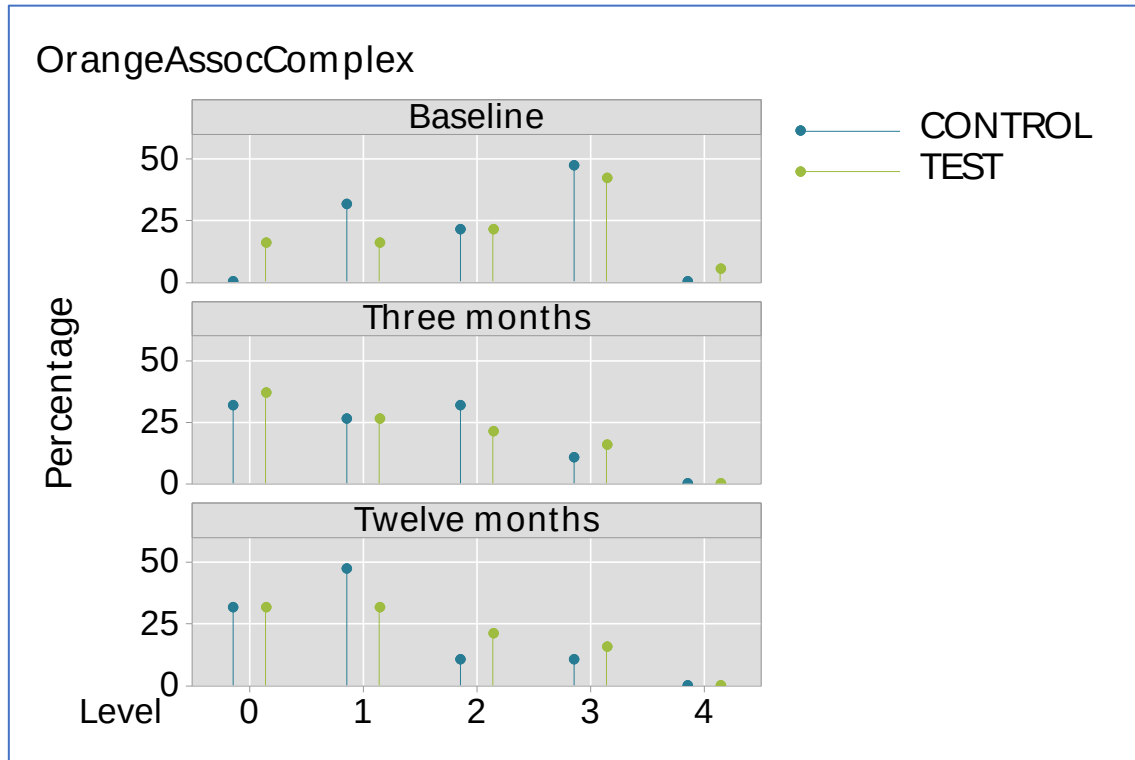
- For level 2 or “*increased bacterial load*”, the control group had an increase in percentage at three months and then a decrease in percentage at twelve months. In the test group, no changes were seen in the percentages at baseline, three and twelve months. It remained at 21.1%.
- Both test and control groups displayed decreases in percentages of level 3 or “*high bacterial load*” of the orange-associated complex, at three months. The percentage for level 3 remained the same at twelve months for both groups.
- The control group had 0% of orange-associated bacteria in the “*extremely high bacterial load*” level (Level 4). However, the test group recorded a 5.3% at baseline and was reduced to 0% at three and twelve months.

Table 4. 27 Percentage of the orange-associated complex at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

Groups	Rank/ Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage Twelve Months	↑/↓/0*
ORANGE ASSOCIATED COMPLEX						
Control	0	0.0	31.6	↑	31.6	0
Test	0	15.8	36.8	↑	31.6	↓
Control	1	31.6	26.3	↓	47.4	↑
Test	1	15.8	26.3	↑	31.6	↑
Control	2	21.1	31.6	↑	10.5	↓
Test	2	21.1	21.1	0	21.1	0
Control	3	47.4	10.5	↓	10.5	0
Test	3	42.1	15.8	↓	15.8	0
Control	4	0.0	0.0	0	0.0	0
Test	4	5.3	0.0	↓	0.0	0

- ↑ - increase in percentage of rank
- ↓- decrease in percentage of rank
- 0- no changes in percentage of rank.

Figure 4. 14 A comparison of percentages for the orange-associated complex at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups



4.3.4.4 Green Complex (*E. corrodens* and *Capnocytophaga sp.*)

Figure 4.15 illustrates the percentage of the green complex at each level and time point for both groups. The following changes occurred in the groups (Table 4.28):

- The percentage of the green complex at level 0 or “*bacterial level below cut off*”, increased post-SRD and at twelve months for the control group. In the test group, an increase was seen at post-SRD and the percentage remained the same at twelve months.
- Increases in percentage of level 1 or “*bacterial levels at cut off*” for the green complex was seen at three months for both groups. At twelve months, the control group remained at the same percentage, whereas the test group increased even further to 42.1%.

- For “*increased bacterial load*” or level 2 of the green complex, decreases were seen at both three and twelve months for the control groups. No changes in percentages were seen in the test group at three months; however further decreases in level 2 of the green complex were noted at twelve months for this group.
- The percentage of level 3 or “*high bacterial load*” of the green complex decreased in control group at three and twelve months. In the control group, 0% was recorded at twelve months. In the test group, decreases in percentages were noted at three months, however this remained the same at twelve months.
- The control group had a 0% of “*extremely high bacterial load*” of the green complex (level 4) at baseline and three months and then increased in percentage at twelve months to 21.1%. No records of green complex at level 4 were seen in the test group.

Table 4. 28 Percentage of the green complex at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups including increase, decrease or no change in percentage

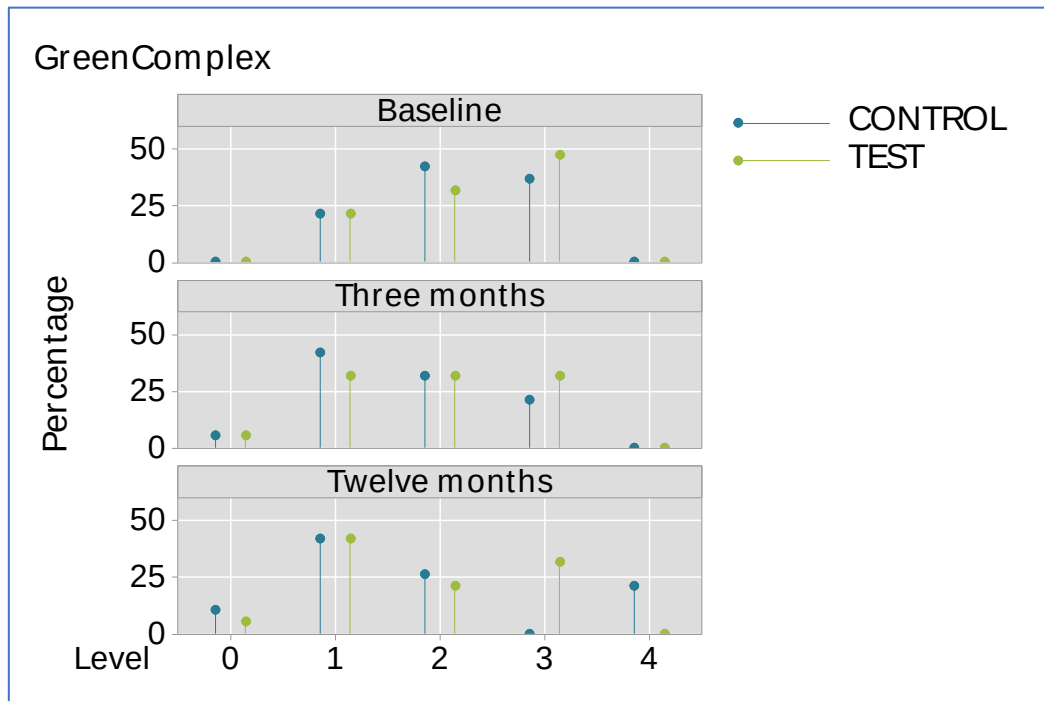
Groups	Rank/ Level	Percentage Pre-SRD	Percentage Post-SRD	↑/↓/0*	Percentage Twelve Months	↑/↓/0*
GREEN COMPLEX						
Control	0	0.0	5.3	↑	10.5	↑
Test	0	0.0	5.3	↑	5.3	0
Control	1	21.1	42.1	↑	42.1	0
Test	1	21.1	31.6	↑	42.1	↑
Control	2	42.1	31.6	↓	26.3	↓
Test	2	31.6	31.6	0	21.1	↓
Control	3	36.8	21.1	↓	0.0	↓
Test	3	47.4	31.6	↓	31.6	0
Control	4	0.0	0.0	0	21.1	↑
Test	4	0.0	0.0	0	0.0	0

* ↑ - increase in percentage

 ↓ - decrease in percentage

 0 - No change

Figure 4. 15 A comparison of percentages for the green complex at each level at baseline (pre-SRD), three months (post-SRD) and at twelve months for control and test groups



4.4 Summary and Discussion of the Findings

Chronic periodontitis is associated with a consortium of bacteria with the initiation and progression of disease often related to the colonization of certain bacteria. Microbiological monitoring can be useful in evaluating efficacy of treatment. The aim of the current study was to examine the subgingival microbiological changes that took place as a result of SRD-only and SRD-with perioscope post-SRD and at twelve months.

The Hain Lifescience Micro-IDent Plus test uses the 16SrRNA PCR method which employs PCR and reverse hybridization (Eick *et al.*, 2011). This test was found to have a good overall agreement in identifying the main periodontal pathogens (Urban *et al.*, 2010) and was used for detection of bacteria for this clinical trial. Pooled samples of five of the deepest pockets were analysed at baseline. Plaque samples were taken of the same sites post-SRD and at twelve months. Bacterial results included information on each pathogen at each level as well as recommended antibiotic therapy for each participant. However, systemic antibiotics were not given to study participants as per the recommendations by Hain. All thirty-eight participants were included in the analysis with no exclusions. When examining the eleven pathogens and mean rank data pre-SRD, post-SRD and at twelve months, no statistically significant differences were detected between groups. However, alterations in pathogen numbers were observed in both groups following therapy and at twelve months. A discussion of these findings will follow.

4.4.1 Pathogen levels pre-SRD for test and control groups

The mean rank data obtained from the Mann Whitney *U* test is an average and allows comparison of pathogens between the test and control groups similar to a T-test. One can infer that a higher mean rank is related to a higher quantity or number of bacteria in that group.

At baseline, five of the eleven pathogens were detected in higher numbers in the test group as compared to the control group including *P. gingivalis*, *P. intermedia*, *P.*

micros, *E. nodatum* and *Capnocytophaga sp.* Six of the eleven pathogens in the control group namely *A. actinomycetemcomitans*, *T. forsythia*, *T. denticola*, *F. nucleatum*, *C. rectus* and *E. corrodens* had higher numbers of bacteria as compared to the test group. The quantity of *T. denticola* and *C. rectus* in the control group was found to be higher than the test group with statistical results bordering on significance. The control group had two of the three pathogens, in the red complex of bacteria, namely *T. denticola* and *T. forsythia* at baseline with higher mean ranks as compared to the test group. One could speculate that the higher numbers of pathogens in the control group at baseline could be a result of the higher mean PPD, BOP and PI in the control group. This is similar to other studies that also found elevated levels of these pathogens in deeper pockets and high levels of inflammation (Gmür *et al.*, 1994; Kigure *et al.*, 1995; Simonson *et al.*, 1992b; Socransky *et al.*, 1998; Socransky *et al.*, 1988; Wara-aswapati *et al.*, 2009).

4.4.2 Changes in Pathogen Levels post-SRD for test and control groups

When examining the mean rank data, the test group had higher numbers of nine of the eleven pathogens as compared to the control group post-therapy. *P. gingivalis*, *P. intermedia* and *Capnocytophaga sp.* were still recorded in higher numbers in the test group post-SRD, however *A. actinomycetemcomitans*, *T. forsythia*, *T. denticola*, *Fusobacterium nucleatum*, *C. rectus* and *E. corrodens* were also observed in higher numbers. The control group had decreases in numbers of these pathogens with only two pathogens namely *P. micros* and *E. nodatum* with higher numbers as compared to the test group. The quantity of both *T. denticola* and *C. rectus* decreased in the control group and were now reported having higher numbers in the test group post-SRD. However no statistically significant differences were found between the two groups.

4.4.3 Changes in Pathogen Levels at Twelve Months for test and control groups.

At twelve months, pathogen levels changed in both groups with the test group having six of the eleven pathogens, namely *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *E. nodatum*, *E. corrodens* and *Capnocytophaga sp.* with higher numbers

as compared to the test group. The quantities of two of the red complex bacteria namely *T. denticola* and *T. forsythia* decreased in the test group and was detected in higher numbers in the control group. The control group at twelve months now had five pathogens (*T. forsythia*, *T. denticola*, *P. micros*, *F. nucleatum*, *C. rectus*) with higher bacterial numbers as compared to the test group. There appeared to be an improvement in quantity of bacteria of pathogens namely *T. forsythia*, *T. denticola*, *F. nucleatum* and *C. rectus* in the test group with mean ranks decreasing as compared to the control group. Only *E. nodatum* decreased in the control group and was found in higher numbers in the test group at twelve months. However, no statistically significant differences in mean ranks between the two groups were established at twelve months.

4.4.4 Effect of SRD-only and SRD-with perioscope on the Eleven Pathogens

Reductions in periodontal microflora following conventional periodontal therapy has been reported in many studies (Cugini *et al.*, 2000; Darby *et al.*, 2005; Darby *et al.*, 2001; Doungudomdacha *et al.*, 2001; Feres *et al.*, 2015; Fonseca *et al.*, 2015; Fujise *et al.*, 2002; Haffajee *et al.*, 1997a, 1997b; Haffajee *et al.*, 2006b; Liu *et al.*, 2013). For the current study, changes in pathogen numbers were detected in both groups post-SRD and at twelve months, with no statistically significant differences was observed between the test and control group. The findings of this study will be compared to literature examining the effects of non-surgical periodontal therapy on periodontal microflora as there is no available studies to date that have examined microbiological changes following SRD with the use of a perioscope.

The prevalence of each pathogen as well change in percentage of the pathogen at each level will provide a more defined understanding of variations to the periodontal microflora at each time point.

4.4.4.1 Effect of SRD-only and SRD-with perioscope on prevalence and levels of *A. actinomycetemcomitans*

Several studies have shown that *A. actinomycetemcomitans* is associated with the aggressive periodontitis (Faveri *et al.*, 2009; Fine *et al.*, 2007; Henderson *et al.*, 2002; Zambon *et al.*, 1983), but has also been found in patients with adult periodontitis (Brigido *et al.*, 2014; Christersson *et al.*, 1992; Grossi *et al.*, 1994; Haffajee *et al.*, 1988b; Holt *et al.*, 2005; Kornman *et al.*, 1991; Mombelli *et al.*, 1994a; Riggio *et al.*, 1996; Rodenburg *et al.*, 1990; Savitt *et al.*, 1984) and patients that are considered periodontally healthy (Fives-Taylor *et al.*, 1999; Slots *et al.*, 1999; Tanner *et al.*, 1998a).

The participants in the current study were diagnosed with moderate to advanced chronic periodontitis. When examining the prevalence of *A. actinomycetemcomitans* pre-SRD, post-SRD and at twelve months in both test and control groups, *A. actinomycetemcomitans* was found below detectable levels in majority of study participants (32 of the 38 (84%)) with only a few participants (6 of 38 (16%)) having *A. actinomycetemcomitans* above threshold levels. Similar results were discovered in the Socransky *et al.*, (1991) study where *A. actinomycetemcomitans* (serotype B) was detected in only 8% of the sampled sites (Socransky *et al.*, 1991b). A lower percentage were reported in the Haffajee *et al.*, (1991) study with only $0.4 \pm 0.8\%$ of *A. actinomycetemcomitans* (serotype b) and $1.1 \pm 1.1\%$ *A. actinomycetemcomitans* (serotype a) found in a sample of 17 patients that were considered to have active disease (Haffajee *et al.*, 1991c). *A. actinomycetemcomitans* was less frequently isolated from chronic periodontitis as compared to localised aggressive periodontitis subjects. In addition, the numbers of *A. actinomycetemcomitans* was not often high as compared to other suspected pathogens taken from the same plaque samples in adults (Rodenburg *et al.*, 1990; Slots *et al.*, 1990).

A. actinomycetemcomitans was predominantly detected at level 0 or “bacterial load under cut off”, pre-SRD, post-SRD and at twelve months, with more than 80% of sites sampled detecting *A. actinomycetemcomitans* at this level. Consideration has to be given to the age of the study population when examining the prevalence and detection of *A. actinomycetemcomitans*. The prevalence of *A. actinomycetemcomitans* has

been found to decrease with age in many studies (Asikainen, 1986; Rodenburg *et al.*, 1990; Asikainen, 1986; Slots *et al.*, 1990). The study conducted in Finnish patients with localised early-onset periodontitis, found *A. actinomycetemcomitans* to be related to age with the presence of *A. actinomycetemcomitans* decreasing with age (Asikainen, 1986). ELISA was used in a total of 5030 plaque samples to determine the presence of *A. actinomycetemcomitans*. Hamlet *et al.*, (2001) stated that *A. actinomycetemcomitans* was found to be the most frequently detected organism (22.8% of subjects), with *A. actinomycetemcomitans* the most frequently detected pathogen in the younger group <30 years as compared to older group 31-40 years old (Hamlet *et al.*, 2001). Low levels of *A. actinomycetemcomitans* were also reported in the study by Darby *et al.*, (2001) where the study population had a mean age of 47($\pm$ 7)years (Darby *et al.*, 2001). Comparably for the current study, the mean age of study population was 52.50 ($\pm$ 11.05) years, with a range of 28-75 years. It is possible that the high prevalence of *A. actinomycetemcomitans* below detectable levels in the current study population could be related to the age of the patients.

4.4.4.1.1 Changes in *A. actinomycetemcomitans* levels post-SRD

Reduction in numbers of *A. actinomycetemcomitans* were observed post-SRD in both groups with a higher percentage of bacteria found below detectable levels (89.47%). This finding is similar to other studies that also reported reduction of *A. actinomycetemcomitans* following SRD (Flemmig *et al.*, 1998a; Listgarten *et al.*, 1991; Maiden *et al.*, 1991; Mombelli *et al.*, 1994a, 1994b; Nieminen *et al.*, 1996; Preber *et al.*, 1995; Renvert *et al.*, 1997; Renvert *et al.*, 1990; Rosenberg *et al.*, 1993; Shiloah *et al.*, 1994; Sigurdsson *et al.*, 1994; Wikström *et al.*, 1993). However, changes in percentages of *A. actinomycetemcomitans* at each level was only minimal, similar to studies that reported minimal changes to *A. actinomycetemcomitans* following therapy (Masunaga *et al.*, 1990).

4.4.4.1.2 Changes in *A. actinomycetemcomitans* levels at twelve months

Recolonization of *A. actinomycetemcomitans* was observed in both groups at twelve months with *A. actinomycetemcomitans* below detectable levels reducing and returning to pre-SRD percentages (84.21%). Majority of study participants still had *A.*

actinomycetemcomitans at levels below threshold; however, an increase in percentages for level 4 or “*extremely high bacterial load*” of *A. actinomycetemcomitans* was recorded for both groups. The test group had a higher percentage (15.79%) as compared to the control group (10.53%). The study by Colombo *et al.*, (2005) also found that *A. actinomycetemcomitans* recolonised and increased to baseline values at nine months after non-surgical periodontal therapy and SPT (Colombo *et al.*, 2005).

Previous studies have discovered that the presence of *A. actinomycetemcomitans* correlates with deep sites (Ebersole *et al.*, 1994; Mombelli *et al.*, 1994a; Slots *et al.*, 1980; Socransky *et al.*, 1991a; Tanner *et al.*, 1979) and is difficult to eradicate from deep periodontal pockets (Bragd *et al.*, 1987; Renvert *et al.*, 1990). Socransky *et al.*, (1991) also found that *A. actinomycetemcomitans* (serotype b) increased with increased pocket depths with mean counts increasing in localised diseased subjects (Socransky *et al.*, 1991b). Likewise, Fujise *et al.*, (2002) also observed increased levels of *A. actinomycetemcomitans* in diseased sites (Fujise *et al.*, 2002). *A. actinomycetemcomitans* was detected in high proportions after treatment in the study by Renvert *et al.*, (1990) and related this with a reduced healing response (Renvert *et al.*, 1990). For the current study, the microbiological test included analyses of pooled samples. Plaque samples were taken from residual deep sites which could have contributed to the increase in numbers of *A. actinomycetemcomitans* at level 4 or “*extremely high load*” at twelve months.

The role of *A. actinomycetemcomitans* in periodontal disease has been ambiguous with some studies showing improvements in clinical outcomes with reductions of *A. actinomycetemcomitans* (Dahlen *et al.*, 1996; Doungudomdacha *et al.*, 2001) whereas some studies showed no association between *A. actinomycetemcomitans* and clinical outcomes (Fujise *et al.*, 2002; Haffajee *et al.*, 1997b; Takamatsu *et al.*, 1999). Even though the association of *A. actinomycetemcomitans* and clinical outcomes was not investigated in this study, the following observations were made. *A. actinomycetemcomitans* reduced post-SRD and improvements in clinical outcomes such as PPD, PAL and BOP were observed in both groups. At twelve months, clinical outcomes improved even further in both groups, however recolonization of *A. actinomycetemcomitans* was detected at this time point. However, the reduction and

recolonization of *A. actinomycetemcomitans* must be interpreted with caution as only low levels of *A. actinomycetemcomitans* were detected in both groups.

4.4.4.2 Effect of SRD-only and SRD-with perioscope on prevalence and levels of *P. gingivalis*

P. gingivalis is part of the red complex of bacteria and its role as an etiological agent in periodontal disease has been investigated and confirmed in numerous studies (Ashimoto *et al.*, 1996; Christersson *et al.*, 1992; Grossi *et al.*, 1994; Haffajee *et al.*, 1988a; Kornman *et al.*, 1991; Mombelli *et al.*, 1991; Mombelli *et al.*, 1995; Moore *et al.*, 1983; Riggio *et al.*, 1996; Riviere *et al.*, 1996; Savitt *et al.*, 1984; Simonson *et al.*, 1992a; Slots, 1979; Socransky *et al.*, 1991a; Socransky *et al.*, 1998; Tanner *et al.*, 1979; Wikström *et al.*, 1993). This pathogen has been reported at higher levels in patients with adult periodontitis in the Australian population. The odds ratio for being positive for *P. gingivalis* was found to be six times higher in PPDs ≥ 3 mm. *P. gingivalis* was found to have a strong association not only with pocket depths but also with mean PAL and mean BOP (Hamlet *et al.*, 2001; O'Brien-Simpson *et al.*, 2000).

The meta-analysis by Rafiei *et al.*, (2018) which included 42 studies published during 1993 to 2016 found that the prevalence of *P. gingivalis* was 78% in periodontal disease groups (Rafiei *et al.*, 2018). The prevalence of *P. gingivalis* was lower for the current study population. Forty-two percent of participants in the control group had *P. gingivalis* detected above threshold levels whilst the prevalence of *P. gingivalis* at this level were reported at 53% in the test group. There were more participants with *P. gingivalis* below threshold levels in the control group (58%) as compared to the test group (47%) pre-SRD. No changes in prevalence of *P. gingivalis* was detected in the test group post-SRD and twelve months. The number of participants below and above threshold levels improved slightly in the control group at both time points. The mean rank data suggest that the test group had a higher quantity of *P. gingivalis* pre-SRD, post-SRD and at twelve months. However, no statistically significant differences were found between the two groups at each time point.

4.4.4.2.1 Changes in *P. gingivalis* levels post-SRD

SRD-only and SRD-with perioscope resulted in decreases in numbers of *P. gingivalis* post-therapy. The control group increased by 5.27% for *P. gingivalis* at level 0 or “bacterial load under cut off” and level 1 or “bacterial load at cut off” and decreased by 10.52% for level 2 or “increased bacterial load”. The test group had reductions in percentages of *P. gingivalis* by 5.26% at both level 3 or “high bacterial loads” and level 4 or “extremely high bacterial loads”.

Reduction of *P. gingivalis* three months post-therapy has been described in many studies (Ali *et al.*, 1992; Haffajee *et al.*, 1997b; Hellstrom *et al.*, 1996; Mombelli *et al.*, 1995; Preber *et al.*, 1995; Renvert *et al.*, 1997; Renvert *et al.*, 1990; Simonson *et al.*, 1992b; Socransky *et al.*, 1993; Wikström *et al.*, 1993). The study by Haffajee *et al.*, (1997) which utilized DNA-DNA hybridization methods to detect periodontal pathogens in a group of 57 patient, found *P. gingivalis* to have reduced significantly three months post-SRD. The percentage of sites colonised post-therapy reduced to 9% for PPDs <4 mm, 15% for PPDs 4-6 mm and 17% for PPDs >6 mm (Haffajee *et al.*, 1997b). The percentage of sites with *P. gingivalis* decreased from 25% to about 12.5% three months post-SRD in the study by Cugini (2000) (Cugini *et al.*, 2000). PCR methods were used to detect levels of *P. gingivalis* post-SRD in the study by Darby *et al.*, (2001). *P. gingivalis* decreased by 10% on a site basis for their study (Darby *et al.*, 2001). Wikström *et al.*, (1993) reported a significant reduction of *P. gingivalis* positive sites from 47.3% before treatment to 15.5% after treatment (Wikström *et al.*, 1993). Reductions in percentage of *P. gingivalis* for this study was slightly lower than the previous studies mentioned when examining *P. gingivalis* at levels 2, 3 and 4 (*P. gingivalis* <10⁵ to *P. gingivalis* >10⁶). A 10.52% reduction was observed in the control group and 5.26% reduction in the test group.

4.4.4.2.2 Changes in *P. gingivalis* levels at twelve months

Improvements in percentage of *P. gingivalis* were observed in both groups at the twelve-month re-evaluation appointment, although no statistically significant differences were observed between the groups. There was an increase in number of *P. gingivalis* at level 0, or “bacterial load under cut off”, with increases in percentages

of 5.26% in both groups. The control group had lower percentages of *P. gingivalis* at level 1 and 2 compared to the test group. The test group had reductions in numbers of *P. gingivalis* at level 3 or “*high bacterial load*” and was able to maintain a 0% level of *P. gingivalis* at level 4 or “*extremely high bacterial load*”. The reduction of *P. gingivalis* reported in this study is comparable to the study by Cugini *et al.*, (2000) who also reported reduction in levels and prevalence of *P. gingivalis* at twelve months. However, statistically significant decreases were observed in their later study (Cugini *et al.*, 2000). Studies by Haffajee *et al.*, (1997) and Colombo *et al.*, (2005) also reported decreases in levels of *P. gingivalis* nine months after therapy (Colombo *et al.*, 2005; Haffajee *et al.*, 1997b). However, *P. gingivalis* was still detected at level 2 or “*increasing bacterial load*” and level 3 or “*high bacterial load*” in both groups despite clinical improvements observed. Likewise, Darby *et al.*, (2001) also reported continuing high prevalence of *P. gingivalis* regardless of clinical improvements (Darby *et al.*, 2001).

The study by Socransky *et al.*, (1991) found that *P. gingivalis* was found more frequently and in higher mean percentages of total viable counts at deeper sites (Socransky *et al.*, 1991b). Comparably other studies have also found that deep persistent pockets are shown to still harbour high levels of this pathogen (Dzink *et al.*, 1988; Mombelli *et al.*, 1995; Mombelli *et al.*, 2000). *P. gingivalis* was significantly elevated in active subjects in the study by Haffajee *et al.*, (1991). Sites that respond poorly to treatment have been found to still harbour this bacteria while sites that resolve, have found *P. gingivalis* at below detectable levels (van Winkelhoff *et al.*, 1988). Similar results were observed in the Fujise *et al.*, (2002) study, with *P. gingivalis* being detected at increased levels in diseased sites (Fujise *et al.*, 2002). One could infer that *P. gingivalis* detectable at level 2 and 3 could be due to the non-resolving sites as both groups had persistent deep pockets from which plaque samples were taken. This could have in turn have affected percentage of *P. gingivalis* recorded for these levels. PPDs sampled ranged from 5.4 mm to 13 mm at baseline; 2.80 mm to 9.4 mm at three months and 2.6 mm to 9.4 mm at twelve months for the study population.

P. gingivalis was not completely eliminated by either of the treatment methods used. Likewise, other studies have reported that even though reduction of *P. gingivalis* in sites has been shown after SRD, complete elimination of the pathogen is not expected

(Danser *et al.*, 1996; Darby *et al.*, 2005; Haffajee *et al.*, 1997b; Haffajee *et al.*, 1995; Haffajee *et al.*, 1988b; Hellstrom *et al.*, 1996; Mombelli *et al.*, 1995; Renvert *et al.*, 1998; Renvert *et al.*, 1990; Shiloah *et al.*, 1994; Simonson *et al.*, 1992b; Socransky *et al.*, 1993; Wikström *et al.*, 1993).

The presence of *P. gingivalis* has been associated with bleeding upon probing (Christersson *et al.*, 1992; Kojima *et al.*, 1993; Mombelli *et al.*, 1991; Socransky *et al.*, 1991b), AL (Dzink *et al.*, 1988; Grossi *et al.*, 1994) and age (Chigasaki *et al.*, 2018; Rodenburg *et al.*, 1990). The analyses of the pathogens involved pooling of samples, and although inferences between detectable levels of *P. gingivalis* with PPD, BOP, AL and age could not be made, the following observations were made. Clinical improvement including reduction of PPDs, improvements in PAL, reduction of BOP were observed in both groups post SRD and at twelve months. *P. gingivalis* also reduced post-SRD and at twelve months.

4.4.4.3 Effect of SRD-only and SRD-with perioscope on prevalence and levels of *T. forsythia*

T. forsythia is part of the red complex bacteria and plays an important role in the pathogenesis of periodontitis. There have been several studies that demonstrated a strong correlation between *T. forsythia* and severity of disease in adult patients (Grossi *et al.*, 1995; Grossi *et al.*, 1994; Tanner *et al.*, 2006a). However, its prevalence varies with some studies reporting a prevalence of 10% (Haffajee *et al.*, 1988c), whilst other studies reporting a prevalence as high as 100% (Christersson *et al.*, 1992). Ashimoto *et al.*, (1996) study utilizing PCR methods found the prevalence of *T. forsythia* to be 86% in patients with advanced periodontitis (Ashimoto *et al.*, 1996). In a more recent study by Mahalakshmi *et al.*, (2018) the prevalence of *T. forsythia* in chronic periodontitis patients was found to be 73.4% (Mahalakshmi *et al.*, 2018). For the current study, the prevalence of *T. forsythia* above threshold levels was 95% in both the test and control groups before commencement of treatment. No changes in prevalence was observed post-SRD in both groups with 95% of participants still harbouring this pathogen above threshold levels. At twelve months, an increase in prevalence of *T. forsythia* was seen in the control group with all participants (100%)

with *T. forsythia* above threshold levels. In the test group, only 84% of the participants had levels of *T. forsythia* above threshold, with *T. forsythia* below threshold levels observed in 16% of participants in this group.

4.4.4.3.1 Changes in *T. forsythia* levels post-SRD

T. forsythia reduced post-SRD in both groups with no statistically significant differences between the test and control groups. From the mean rank data, the control group had higher numbers of *T. forsythia* pre-SRD as compared to the test group, with the difference between the groups bordering on significance. *T. forsythia* was detected mostly at level 3 or “*high bacterial load*” in both groups with only minimal amounts of *T. forsythia* found at other levels. *T. forsythia* reduced in the control group post-SRD with higher numbers now found in the test group. Many studies have reported a decrease in *T. forsythia* following SRD (Darby *et al.*, 2005; Darby *et al.*, 2001; Haffajee *et al.*, 1997b; Takamatsu *et al.*, 1999; Teles *et al.*, 2006; Van der Velden *et al.*, 2003). Takamatsu *et al.*, (1999) utilised DNA probes to determine the effects of SRD on prevalence of *T. forsythia* on 26 patients with periodontitis. Participants were evaluated one month post-therapy and it was reported that frequency and detection of *T. forsythia* was significantly reduced (Takamatsu *et al.*, 1999). The study by Cugini *et al.*, (2000) used checkerboard DNA-DNA hybridization methods to determine the presence and levels of the 40 subgingival species. This study reported a reduction of *T. forsythia* following SRD with the most reduction occurring three months post-SRD (Cugini *et al.*, 2000). Darby *et al.*, (2005) found a significant decrease in percentage of *T. forsythia* (36.3%) in 28 chronic periodontitis subjects 6-8 weeks following therapy (Darby *et al.*, 2005). The reduction of *T. forsythia* post-SRD in the current study was lower than the Darby *et al.*, (2005) study with the percentage decrease at level 3 or “*high bacterial load*” in the control group recorded at 21.05% and in the test group, 15.79%.

Increases in *T. forsythia* were noted in both groups for level 4 or “*extremely high bacterial load*” of *T. forsythia*, with the control group increasing by 5.27% and the test group by 15.79%. *T. forsythia* has been found to increase in numbers in sites with increasing pocket depths (Christersson *et al.*, 1992; Dzink *et al.*, 1985; Machtei *et al.*, 1997a; Socransky *et al.*, 1991b). Research conducted in the past have found that high levels of *T. forsythia* were found more commonly in diseased sites as compared to

healthy sites (Haffajee *et al.*, 1998; Ximenez-Fyvie *et al.*, 2000a) with *T. forsythia* been strongly associated with active or progressing lesions (Dzink *et al.*, 1983; Dzink *et al.*, 1988; Grossi *et al.*, 1994; Tanner *et al.*, 1989; Tanner *et al.*, 1998a; Tanner *et al.*, 1987b). Microbiological samples were pooled with persistent deep pockets still present post-SRD in both groups. Presumably, the increases in percentage of *T. forsythia* for “*extremely high bacterial load*” could be a result of plaque samples taken from pockets that are deep and likely active.

Studies have reported a strong association between *P. gingivalis* and *T. forsythia*. Gmür *et al.*, (1989) found that samples positive for *P. gingivalis* were also positive for *T. forsythia* (Gmür *et al.*, 1989). The relationship of these bacteria was supported by Lotufo *et al.*, (1994) who reported that *P. gingivalis* and *T. forsythia* were found together in 55% of sites (Lotufo *et al.*, 1994). The co-existence of *P. gingivalis* and *T. forsythia* was examined using colorimetric PCR methods in the Fujise *et al.*, (2002) study and it was found that SRD reduced the levels and co-colonization from 75% of sites to 43% of sites three months post-therapy (Fujise *et al.*, 2002). For the current study, both *P. gingivalis* and *T. forsythia* reduced post-therapy in both groups. However, co-colonization of these bacteria was not examined in this study.

4.4.4.3.2 Changes in *T. forsythia* levels at twelve months

When examining the mean rank data at twelve months, the test group had lower numbers of *T. forsythia* as compared to the control group. An increase in percentage of *T. forsythia* at level 0 or “*bacterial level below cut off*” and a reduction of percentage of *T. forsythia* at level 3 or “*high bacterial load*” (42.11%) was recorded in the test group. The control group had 68.42% of *T. forsythia* at level 3 at twelve months. One can infer that the test group was more effective in reducing numbers of *T. forsythia* at twelve months as compared to control group; however, no statistically significant differences were found between the groups. The increase in percentage of *T. forsythia* at level 3 in the control group is similar to the Cugini *et al.*, (2000) study who also found that slight increases in *T. forsythia* was seen at 9 and 12 months (Cugini *et al.*, 2000).

T. forsythia at level 4 or “*extremely high bacterial load*” remained unchanged in both groups. As suggested before, the analysis of pooled samples with deep persistent pockets could have influenced this finding.

4.4.4.4 Effect of SRD-only and SRD-with perioscope on prevalence and levels of *T. denticola*

T. denticola has been reported more frequently in periodontally diseased sites than healthy sites with this pathogen also part of the red complex of bacteria (Haffajee *et al.*, 1998; Riviere *et al.*, 1992; Simonson *et al.*, 1988; Socransky *et al.*, 1998). Similarly, for this study *T. denticola* was found at high prevalence above threshold levels in both groups (100% in the control group, 95% in the test group). Changes in prevalence were observed post-SRD with reduction in prevalence above threshold in both groups to 79% and an increase in prevalence below threshold to 11%. At twelve months, the prevalence of *T. denticola* improved in the test group with more participants having *T. denticola* below threshold levels (from 11% post-SRD to 26% at twelve months). No further changes were observed in the control group at twelve months.

4.4.4.4.1 Changes in *T. denticola* levels post-SRD

T. denticola was detected in higher numbers in the control group as compared to the test group pre-SRD. Post-treatment resulted in reduction of numbers of *T. denticola*, with the control group doing much better in lowering numbers of *T. denticola* as compared to the test group. Likewise, many studies have also reported reductions of *T. denticola* at three months following SRD (Cugini *et al.*, 2000; Darby *et al.*, 2001; Haffajee *et al.*, 1997b; Simonson *et al.*, 1992b). The results obtained from the DNA-DNA checkboard analysis found that *T. denticola* decreased significantly in prevalence and levels three months following therapy (Haffajee *et al.*, 1997b). On a site basis, significant reductions in *T. denticola* of about 20% were observed in the Darby *et al.*, (2001) study post-SRD (Darby *et al.*, 2001). For the current study, the control group had a 21.06% reduction of *T. denticola* at level 3 or “*high bacterial load*” with the test group having a 15.78% reduction at level 2 or “*increasing bacterial load*”.

T. denticola was still detectable at level 2 or “increasing bacterial load” and level 3 or “high bacterial load” in both groups. Previous studies have found that *T. denticola* was detected more frequently in periodontally diseased sites than healthy sites (Riviere *et al.*, 1997; Simonson *et al.*, 1988). The levels of *T. denticola* were found to decrease in treated sites with only minimal changes seen in non-responding sites (Simonson *et al.*, 1992b). For this study population, pooled samples were taken which included deep sites which could be diseased or non-responding which in turn could have influenced the percentage of *T. denticola* at these levels.

4.4.4.4.2 Changes in *T. denticola* levels at twelve months

Recolonization of *T. denticola* was not evident at twelve months and levels of *T. denticola* were similar to that recorded post-SRD. *T. denticola* percentages reduced even further at level 3 for both groups, with the test group having a 21.05% reduction and the control group a 5.26% reduction in *T. denticola* at this level. The study by Cugini *et al.*, (2000) also found that *T. denticola* decreased in prevalence and levels up to six months and remained at these levels at 9 and 12 months (Cugini *et al.*, 2000). When examining the prevalence data for *T. denticola* below and above threshold levels, the test group recorded a decrease in number of participants from 17 (89%) to 14(74%) from post-SRD to twelve months and an increase in the number of participants from 2 (11%) to 5 (26%) for *T. denticola* below threshold levels. No changes in prevalence was noted for the control group.

Studies have shown that *T. denticola* is correlated with decreasing pocket depths and BOP (Cugini *et al.*, 2000; Darby *et al.*, 2005; Simonson *et al.*, 1988). A significant decrease in *T. denticola* levels in sites with clinical improvement were also reported in the Haffajee *et al.*, (1997) (Haffajee *et al.*, 1997a). For the current study both groups had reduction of mean PPD and mean BOP post-SRD and at twelve months, with the test group having a significantly lower mean PPD and BOP than the control group at twelve months. *T. denticola* reduced at both time points with more reductions noted at twelve months in the test group. However, correlation analysis comparing clinical and microbiological outcomes were not included. *T. denticola* detected at level 2 or “increasing bacterial load” and level 3 or “high bacterial load” at twelve months in both

groups could be related to the pooling of samples and microbiological analysis including remaining deep sites.

Studies have demonstrated that *T. denticola* has been found to interact more especially with *P. gingivalis* (Grenier, 1992; Onagawa *et al.*, 1994). In a study using immune-histochemical methods *T. denticola* was found to be co-existing with *P. gingivalis* in deeper pockets (Kigure *et al.*, 1995). For this study, reductions in *P. gingivalis* and *T. denticola* were observed, however both pathogens were still detectable at levels above threshold.

4.4.4.5 Effect of SRD-only and SRD-with perioscope on prevalence and levels of *P. intermedia*

Studies have reported an increase in prevalence of *P. intermedia* in patients with adult periodontitis (Ali *et al.*, 1997; Christersson *et al.*, 1992; Dahlen *et al.*, 1992a; Kornman *et al.*, 1991; Mombelli *et al.*, 1991; Moore *et al.*, 1983; Rodenburg *et al.*, 1990; Savitt *et al.*, 1984; Slots *et al.*, 1990; Socransky *et al.*, 1991a; Wikström *et al.*, 1993). However, the prevalence differed between studies with some reporting high prevalence and others not. The prevalence of *P. intermedia* was found to be 63% in the untreated patient group and 59% in patients with refractory disease in the Rodenburg *et al.*, (1990) study (Rodenburg *et al.*, 1990). Christersson *et al.*, (1992) used immunofluorescence to detect the distribution of *P. intermedia* and reported that *P. intermedia* was detected in one or more sites of all patients (100% prevalence) (Christersson *et al.*, 1992). For the current study, *P. intermedia* was detected below threshold levels for majority of the participants with only a few participants having *P. intermedia* above threshold levels. The prevalence of *P. intermedia* reported for this study is similar to that of the study by Savitt and Socransky (1984), where the frequency of detection of *P. intermedia* was 29% in patients with adult periodontitis (Savitt *et al.*, 1984). Twenty six percent of participants in the control group and 37% in the test group had *P. intermedia* above threshold levels. Post-SRD saw only minimal changes in prevalence of *P. intermedia*; however, at twelve months, the prevalence of *P. intermedia* below threshold levels increased in both groups. The mean rank data

suggests that *P. intermedia* was detected in higher numbers in the test group pre-SRD, post-SRD and at twelve months, although no statistically significant differences were reported between groups.

4.4.4.5.1 Changes in *P. intermedia* levels post-SRD

Both SRD-only and SRD-with perioscope resulted in decreases in *P. intermedia* post-SRD. The percentage of *P. intermedia* at level 0 or “bacterial levels below cut off” still remained high for both groups. The SRD-with perioscope group has a higher percentage of *P. intermedia* at levels “below cut off” and a decrease in *P. intermedia* at level 3 or “high bacterial load” and level 4 “extremely high bacterial load”. The control group had improvements in percentages of *P. intermedia* at level 2 or “increasing bacterial load”. This finding is similar to studies in the past which also reported a decrease in *P. intermedia* after SRD (Ali *et al.*, 1992; Bragd *et al.*, 1987; Colombo *et al.*, 2005; Renvert *et al.*, 1990; Socransky *et al.*, 1993; van Winkelhoff *et al.*, 1988; Wikström *et al.*, 1993). Nevertheless, only minimal differences were detected similar to the study by Loesche *et al.*, in 1985 who reported no effect of *P. intermedia* levels after SRD (Loesche *et al.*, 1985).

4.4.4.5.2 Changes in *P. intermedia* levels at twelve months

At twelve months, levels of *P. intermedia* remained low and did not return to the pre-SRD levels. However, only negligible changes were seen at twelve months with no statistically significant differences between the groups. *P. intermedia* was still mostly detected at level 0 or “bacterial levels below cut off”.

There have been conflicting views with the association of *P. intermedia* with pocket depths (Colombo *et al.*, 2002; Doungudomdacha *et al.*, 2000; Dowsett *et al.*, 2002); as well as levels of *P. intermedia* between active and inactive sites (Haffajee *et al.*, 1997b; Haffajee *et al.*, 1991c; Tanner *et al.*, 1998a). The prospective study by Brown *et al.*, (1992) as well as the retrospective study by Dzink *et al.*, (1998), reported increases in levels of *P. intermedia* to be associated with disease activity (Brown *et al.*, 1993; Dzink *et al.*, 1988). It is difficult to infer whether the increase in percentage of *P. intermedia*

at level 3 or “*high bacterial load*” at twelve months could be linked to disease activity. There were residual deep pockets that were sampled in both groups and whether this contributed to the increase in percentage of *P. intermedia* at this level cannot be determined.

4.4.4.6 Effect of SRD-only and SRD-with perioscope on prevalence and levels of *P. micros*

P. micros is a gram positive anaerobic coccus which has been implicated in the pathogenesis of periodontal disease in past studies (Dzink *et al.*, 1988; Moore *et al.*, 1983; Moore *et al.*, 1982; Rams *et al.*, 1992; Tanner *et al.*, 1979; Yasui, 1989). It has been reported more frequently in higher numbers in adult periodontitis (Moore *et al.*, 1983). The prevalence of *P. micros* in advanced adult periodontitis were reported as 58% to 63% in the study by Rams *et al.*, 1992 (Rams *et al.*, 1992). Participants in the current study were diagnosed with chronic moderate to advanced periodontal disease. When examining the prevalence data, all participants (100%) had detectable levels of *P. micros* before commencement of treatment. The prevalence remained unchanged in the control group following therapy, with the test group having 16% of participants with *P. micros* below threshold levels. At twelve months, 2 of the 19 participants in the test group had *P. micros* below threshold levels compared to 1 of 19 in the control group.

4.4.4.6.1 Changes in *P. micros* levels post-SRD

P. micros reduced after SRD in both groups. The mean rank data suggest that the control group had higher numbers of *P. micros* than the test group post-SRD. However, these differences were not statistically significant. There was an almost 50% increase for *P. micros* at level 2 or “*increased bacterial load*” documented post-SRD in the control group. *P. micros* has been isolated in higher frequencies and numbers in active periodontal sites or sites with periodontal destruction (Dzink *et al.*, 1988; Haffajee *et al.*, 1991a; Moore *et al.*, 1991; Rams *et al.*, 1992; Tanner *et al.*, 1987a). However, this finding could be incidental as both groups had persistent pockets from which plaque samples were taken.

4.4.4.6.2 Changes in *P. micros* levels at twelve months

No significant differences were found between groups with regards to levels of *P. micros* at twelve months. The mean rank data suggests that the test group had lower numbers of *P. micros* at twelve months as compared to the control group. After continued SPT, more reduction of numbers of *P. micros* occurred in test group with some recolonization occurring at level 3 or “*high bacterial load*” for the control group. Eleven percent of participants had *P. micros* “*below threshold levels*” in the test group compared to 5 % in the control group.

P. micros was reduced but not eliminated by either of the treatment modalities with *P. micros* still detected at level 2 or “*increasing bacterial load*” and level 3 or “*high bacterial load*” in both groups. The percentage of *P. micros* at level 2 and level 3 was 78.95% in the control group and 57.89% in the test group at twelve months which is higher than the Rams *et al.*, (1992) study. Using pooled samples *P. micros* was identified in 26.7% of participants in active sites at six months and 53.3% at twelve months in their study. The prevalence of *P. micros* was higher in maintenance patients with active disease suggesting the role of this bacteria in progressive AL (Rams *et al.*, 1992). Haffajee *et al.*, (1998) also found *P. micros* to be associated with progression of disease (Haffajee *et al.*, 1988a). For the current trial, samples were pooled and there were remaining deep pockets at the end of this clinical trial, suggesting that there could be residual active sites or sites that could progress even further.

4.4.4.7 Effect of SRD-only and SRD-with perioscope on prevalence and levels of *F. nucleatum*

F. nucleatum is considered a ‘bridge bacterium’ by means of its support of growth of other anaerobic bacteria (Bolstad *et al.*, 1996). It is prevalent in subjects with periodontitis (Boutaga *et al.*, 2006; Colombo *et al.*, 2002; Papapanou *et al.*, 2000; Socransky *et al.*, 2002b) and was found to reduce after successful periodontal therapy in some studies (Haffajee *et al.*, 2006b; Van der Velden *et al.*, 2003).

For the current study, all participants (100%) in the control group had *F. nucleatum* detected above threshold levels, whilst 95% of participants in the test group recording

F. nucleatum at these levels. The prevalence of *F. nucleatum* in the current study is similar to studies that have found *F. nucleatum* occurring in 80-100% of adult periodontitis patients (Lippke *et al.*, 1991; Mombelli *et al.*, 1995; Papapanou *et al.*, 1993; Savitt *et al.*, 1984; Slots *et al.*, 1991; Wikström *et al.*, 1993). No changes in prevalence was noted post-SRD in both groups. At twelve months *F. nucleatum* was found in all participants (100%) in the test and control group above threshold levels.

4.4.4.7.1 Changes in levels of *F. nucleatum* post-SRD

F. nucleatum was detected at higher numbers in the control group pre-SRD. Post-SRD resulted in the test group having higher numbers of *F. nucleatum*, though no statistically significant differences were found between the two treatment groups.

Post-SRD resulted in reductions of percentages at level 2 or “*increased bacterial load*” for both groups. However, for level 3 or “*high bacterial load*”, increases in percentages were observed for both groups, with *F. nucleatum* detected at higher percentages (84.21%) in the test group as compared to the control group (73.68%). These results are similar to the studies by Wikström *et al.*, (1993) and Haffajee *et al.*, (1997) who also reported an increase in *F. nucleatum* after SRD (Haffajee *et al.*, 1997b; Wikström *et al.*, 1993). Colombo *et al.*, (2005) also found that *F. nucleatum* increased in frequency and counts after treatment (Colombo *et al.*, 2005).

Deep persistent pockets were included in the pooled analysis of plaque samples. *F. nucleatum* has been correlated with pocket depth (Dzink *et al.*, 1985) and has also been found isolated in active sites (Dzink *et al.*, 1988; Moore *et al.*, 1991; Tanner *et al.*, 1987b). The increase in percentage of *F. nucleatum* at level 3 or “*high bacterial load*” could be related to the deep pockets that remained post-SRD.

4.4.4.7.2 Changes in levels of *F. nucleatum* at twelve months

At the twelve-month appointment, *F. nucleatum* was found in higher numbers in the control group as compared to the test group, as observed by the mean rank data, however no statistically significant differences were observed between the two groups. *F. nucleatum* was detected above threshold levels in all participants in both groups at

twelve months. Increases in percentages were observed for level 2 or “*increasing bacterial load*” for both groups. The study by Cugini *et al.*, in 2000, also reported increases in levels and prevalence of *F. nucleatum* at twelve months (Cugini *et al.*, 2000). However, for level 3 or “*high bacterial load*”, reductions in percentages of *F. nucleatum* were seen with percentages back to pre-SRD percentages. A similar finding was reported in the Haffajee *et al.*, (2006) study where *F. nucleatum* returned to baseline values at twelve months (Haffajee *et al.*, 2006b).

4.4.4.8 Effects of SRD-only and SRD-with perioscope on prevalence and levels of *C. rectus*

There have been mixed views with regards to *C. rectus* and its role in periodontal disease. *C. rectus* was thought to be more an opportunistic pathogen, found in both healthy and subjects with periodontal disease and has not been strongly associated with periodontal disease progression (Gmür *et al.*, 1994). In the study by Mayanagi *et al.*, (2004), *C. rectus* was found in both healthy and diseased sites with over 80% of both groups harbouring this pathogen (Mayanagi *et al.*, 2004). Gmür and Guggenheim (1994) also reported 40% of *C. rectus* in supragingival plaque in periodontally healthy subjects (Gmür *et al.*, 1994). However, studies by Dzink *et al.*, (1985) and Rams *et al.*, (1993) found *C. rectus* in higher numbers and frequency in sites of active destruction (Dzink *et al.*, 1985; Rams *et al.*, 1993). The prevalence of *C. rectus* for the current study was higher for *C. rectus* above threshold levels for both groups with the control group having a 100% prevalence of *C. rectus* at this level and the test group, 84% before the start of treatment. No changes in prevalence was observed in the test group post-SRD. The control group on the other hand had more participants having *C. rectus* below threshold levels (21%). At twelve months, the test group prevalence of *C. rectus* below threshold levels increased from 3 (16%) post-SRD to 7 (37%) at twelve months. In the control group, an increase of prevalence from 4 (21%) to 5 (26%) was observed. The observation is the test group had more participants below threshold levels than the control group following SPT appointments.

4.4.4.8.1 Changes in levels of *C. rectus* post-SRD

For this study, the numbers of *C. rectus* were found to be higher in the control group pre-SRD as compared to the test group more especially for level 3 or “*high bacterial load*” of *C. rectus*. Post-SRD showed decreases in percentage of *C. rectus* in both groups with the control group having more reduction of this pathogen at this level. Similar results were observed in the study by Wikström *et al.*, (1993) who found that the total variable count of *C. rectus* reduced post-therapy (Wikström *et al.*, 1993) and Rams *et al.*, (1993) who reported a decrease of *C. rectus* from 8.2% to 0.7% following local periodontal debridement of 20 culture positive adult periodontitis patients (Rams *et al.*, 1993). Colombo *et al.*, (2005) also reported significant reductions in levels and prevalence of *C. rectus* three months post therapy (Colombo *et al.*, 2005).

4.4.4.8.2 Changes in levels of *C. rectus* at twelve months

At twelve months, both groups had reductions of percentages of *C. rectus* at level 2 or “*increased bacterial loads*”. However, recolonization did occur with percentages at level 3 or “*high bacterial load*” increasing in both groups but not reaching pre-SRD percentages. Both Tanner *et al.*, (1987) and Haffajee *et al.*, (1988) detected elevated levels of *C. rectus* in progressive active or non-responding sites (Haffajee *et al.*, 1988a; Tanner *et al.*, 1987b). The high percentage of *C. rectus* at twelve months could be linked to the deep persistent pockets that were analysed in a pooled sample for both groups. Both Lai *et al.*, (1992) and Haffajee *et al.*, (1991) inferred that *C. rectus* can be linked to the disease recurrence (Haffajee *et al.*, 1991c; Lai *et al.*, 1992) suggesting that the levels of *C. rectus* be closely monitored.

4.4.4.9 Effects of SRD-only and SRD-with perioscope on prevalence and levels of *E. nodatum*

In 2008, Haffajee *et al.*, found the presence of *E. nodatum* with the red complex bacteria in supragingival plaque samples in subjects with periodontitis (Haffajee *et al.*, 2008b). *E. nodatum* was found in lower proportions in health and gingivitis but in higher proportions in subjects with different periodontal conditions in studies conducted by Moore *et al.*, (1982) and (1985) (Moore *et al.*, 1985; Moore *et al.*, 1982). This was

similar finding in the study by Papapanou *et al.*, (2000) who found higher counts of *E. nodatum* in the 134 periodontitis patients (Papapanou *et al.*, 2000).

The prevalence of *E. nodatum* above threshold was 74% in the control group and 79% in the test group. Post-SRD resulted in decrease in *E. nodatum* above threshold levels to 58% in the control group and 47% in the test group. Further decreases in prevalence of *E. nodatum* for this level was seen in the control group (42%), and a slight increase to 53% was observed in the test group.

4.4.4.9.1 Changes in level of *E. nodatum* post-SRD

Decreases in *E. nodatum* percentages were noted for both groups post-SRD especially for level 2 or “*increasing bacterial load*” and level 3 or “*high bacterial load*”, although no significant differences were found between groups. The study by Haffajee *et al.*, (1997), found only a slight decrease in *E. nodatum* after SRD (Haffajee *et al.*, 1997b). In a more recent study by Feres *et al.*, (2015), using the DNA-DNA checkerboard hybridization technique, which evaluated 38 species in smokers and non-smokers, a significant reduction in mean counts of *E. nodatum* at Day 0 and 63 days post-SRD in non-smokers was observed (Feres *et al.*, 2015).

4.4.4.9.2 Changes in levels of *E. nodatum* at twelve months

E. nodatum percentages at each level continued to improve in both groups at twelve months. The control group had a higher prevalence of *E. nodatum* below threshold levels (58%) as compared to 47% in the test group. *E. nodatum* detected at level 0 or “*bacterial load under cut off*” was higher in the control group as compared to the test group. However, no statistically significant differences were found between the two groups at twelve months.

Using DNA-DNA hybridization method in 24 untreated Brazilian subjects with chronic periodontitis, Colombo *et al.*, (2009) found a positive correlation between *E. nodatum* and mean pocket depth and attachment levels (Colombo *et al.*, 2009). For the current study, both treatment groups displayed decreases in mean pocket depths and improvements in mean clinical attachment levels post SRD and at twelve months.

However, small percentages of *E. nodatum* was still found at level 2 or “increased bacterial load” and level 3 or “high bacterial load” in both groups suggesting that the deeper pockets could still be harbouring *E. nodatum* above threshold levels. This is similar to the study by Booth *et al* in 2004 who found *E. nodatum* to be at higher levels in deeper pockets (Booth *et al.*, 2004).

4.4.4.10 Effect of SRD-only and SRD-with perioscope on prevalence and levels of *E. corrodens*

E. corrodens has been associated with periodontal disease in some but not all studies. In the study by Savitt and Socransky (1984), *E. corrodens* was found be more prevalent and at higher levels in patients with gingivitis and periodontal disease as compared to healthy subjects. In addition, no significant difference was found between gingivitis and periodontitis sites (Savitt *et al.*, 1984). This pathogen was detected at statistically higher levels in periodontal disease than in healthy subjects, with 67% reported in healthy subjects and 94% in periodontitis subjects (Suda *et al.*, 2002). Similarly, Chen *et al.*, (1989) also detected *E. corrodens* in 70% of healthy subjects but in all subjects with periodontal disease (100%) (Chen *et al.*, 1989).The prevalence of *E. corrodens* for the current study is comparable to these studies as *E. corrodens* was found above threshold levels in 100% of the participants in the control group and 89% in the test group. Post-SRD resulted in some changes to prevalence in the groups, with the control group’s prevalence reducing to 89% and the test group increasing to 95%. Further reductions in prevalence of *E. corrodens* above threshold levels were observed in both groups with the control group reducing to 79% and the test group to 84% at twelve months.

4.4.4.10.1 Changes in level of *E. corrodens* post SRD

Decreases in *E. corrodens* was observed post-SRD in both groups. However, treatment resulted in only minimal decreases. This is similar to the study by Haffajee *et al.*, (1997) who reported little change to prevalence and numbers of *E. corrodens* after SRD (Haffajee *et al.*, 1997b).

4.4.4.10.2 Changes in level of *E. corrodens* at twelve months

E. corrodens numbers continued to decrease at twelve months in both groups. However, *E. corrodens* still remained at approximately 50% for both groups at twelve months for level 2 or “increasing bacterial loads” of *E. corrodens*. *E. corrodens* was detected more frequently in active lesions by Dzink *et al.*, in (1995) (Dzink *et al.*, 1985) as did Tanner *et al.*, in 1987 (Tanner *et al.*, 1987a). *E. corrodens* was also found in subjects who responded poorly to periodontal treatment (Haffajee *et al.*, 1988b) and in the Suda *et al.* study in 2002, this bacterium was found to be elevated in lesions in subjects with localized aggressive periodontitis (Suda *et al.*, 2002). The high quantities of *E. corrodens* at “increasing bacterial load” could be a result of the remaining deep pockets. This could infer that active areas and areas that responded poorly to treatment were still present.

4.4.4.11 Effect of SRD-only and SRD-with perioscope on prevalence and levels of *Capnocytophaga sp.*

Capnocytophaga sp. are thought to be early colonizers together with *A. viscosus* and *Streptococcal* species and with other species may be implicated in the formation of the orange and then red complex bacteria (Socransky *et al.*, 1998). *C. gingivalis* was found to be in healthy patients in the study by Haffajee *et al.*, (2006) (Haffajee *et al.*, 2006a)

The prevalence of *Capnocytophaga sp.* varied in studies. Papapanou *et al.*, (1993) reported that 32% of the subjects were positive for *Capnocytophaga* (Papapanou *et al.*, 1993). The study by Wikström *et al.*, (1993) found the prevalence of *Capnocytophaga sp.* positive sites to be 63.3% before treatment (Wikström *et al.*, 1993). The prevalence of *Capnocytophaga* species was found to be 60% in adult periodontitis in the study by Kornman *et al.*, (1991) (Kornman *et al.*, 1991).

The prevalence of *Capnocytophaga sp.* was higher for this study. *Capnocytophaga sp.* for above threshold levels pre-SRD was 84% in the control group and 95% in the test groups. There were no changes in prevalence of *Capnocytophaga sp.* in both groups for above and below threshold levels of *Capnocytophaga sp.* post-SRD and twelve

months. For the current study, higher numbers of *Capnocytophaga sp.* were seen in the test group pre-SRD, post-SRD and at twelve months when compared to the control group, when examining mean rank data, although no statistically significant differences were found.

4.4.4.11.1 Changes in *Capnocytophaga sp.* levels post-SRD

The percentage of *Capnocytophaga sp.* decreased at level 2,3, and 4 in the control group post-SRD. The test group had decreases in percentage of *Capnocytophaga sp.* at level 3 or “*high bacterial load*”. Ali *et al.*, (1992) also found that *Capnocytophaga sp.* reduced after therapy (Ali *et al.*, 1992).

4.4.4.11.2 Changes in *Capnocytophaga sp.* levels at twelve months

Recolonization of *Capnocytophaga sp.* occurred at twelve months with increases in percentages of *Capnocytophaga sp.* at level 2 or “*increasing bacterial load*” and level 3 or “*high bacterial load*” in both groups. Increases in levels and prevalence of *Capnocytophaga sp.* at twelve months was also described in the study by Cugini *et al.*, in 2000 who used a similar study protocol of taking samples pre-SRD and at three, six, nine and twelve months. However, samples were taken from individual sites and were not pooled (Cugini *et al.*, 2000).

4.4.5 Effect of SRD-only and SRD-with perioscope on the bacterial complexes

It has been established over the years that bacterial species exist in complexes in subgingival plaque and are involved with periodontal disease progression (Socransky *et al.*, 1998). The three pathogens, namely *P. gingivalis*, *T. denticola* and *T. forsythia*, were analysed as part of the red complex bacteria. The pathogens in the orange complex included *P. intermedia*, *P. micros*, *F. nucleatum*. For the orange- associated complex (*C. rectus* and *E. nodatum*) were examined and the green complex included *E. corrodens* and *Capnocytophaga sp.*

No statistical differences in bacterial numbers between the test and control groups were established for the red, orange, orange-associated and green complexes of

bacteria at baseline, post-SRD or twelve months. The mean rank data indicated that pathogens in the red and orange-associated complexes were found in higher numbers in the control group at baseline as compared to the test group. The orange and green complexes had higher numbers in the test group as compared to the control group at baseline.

Post-SRD showed changes in mean ranks with the test group having higher number of samples with higher levels for red, orange and green complexes, whilst only higher numbers of bacteria in the orange-associated complex were reported in the control group. This may infer that post-SRD, the SRD-only group was more effective in reducing the red, orange and green complexes of bacteria as compared to the test group, whilst the test group was effective in reducing numbers of pathogens in the orange-associated complex.

At twelve months, the mean ranks of the complexes of bacteria altered again with the test group doing much better in reducing numbers of bacteria in the red and orange complex as compared to the control group. The bacteria in the orange-associated and green complex bacteria were reported at lower numbers in the control group. This could suggest that the SRD-with perioscope group had more reduction in the number of bacteria in the red and orange complex at twelve months as compared to the control group. The control group, on the other hand was better in reducing the numbers of bacteria in the orange-associated and green complex.

The analysis of the percentage of the complexes of bacteria at each level provided a more detailed understanding of changes in numbers of pathogens in their complexes at each time point.

4.4.5.1 Effect of SRD-only and SRD-with perioscope on the red complex

Bacteria in the red complex (*P. gingivalis*, *T. forsythia* and *T. denticola*) are known to be the primary causative periodontal pathogens with strong to very strong pathogenicity (Socransky *et al.*, 1998). Increased counts, proportions and prevalence of the red complex bacteria were found in subjects with periodontal disease (Haffajee

et al., 2006b; Ximenez-Fyvie *et al.*, 2000a). This complex of bacteria were pre-dominant pathogens of moderate to severe form of chronic periodontitis and significantly associated with disease severity in the study by Hamlet *et al.*, (2001) (Hamlet *et al.*, 2001). Comparably for this study population diagnosed with moderate to advanced chronic periodontitis, the red complex bacteria were detected at high percentages for level 2 or “*increased bacterial loads*” and level 3 or “*high bacterial loads*” pre-therapy. The study by da Silva-Boghossian *et al.*, (2011) found in patients with chronic periodontitis, the mean prevalence of the red complex was about 50% (da Silva-Boghossian *et al.*, 2011). Percentage of the red complex was higher for this study population especially at level 2 or “*increased bacterial load*” (63.2% in the control group and 52.6% in the test group) and for level 3 or “*high bacterial load*”, the detection of the red complex was 31.6% in both groups. The red complex was detected in higher numbers in the control group pre-SRD. This complex of bacteria has been associated with probing depths and BOP (Socransky *et al.*, 2002a; Socransky *et al.*, 1998). The control group had higher mean PPDs, BOP and PI pre-therapy which could be related to the higher number of red complex bacteria.

4.4.5.1.1 Changes on levels of red complex bacteria post -SRD

Post-SRD, improvements in percentages of the red complex, with a reduction in percentage for level 3 or “*high bacterial loads*” and an increase in percentage of level 0 or “*bacterial load under cut off*” were observed in the control group. SRD-only resulted in reduction of the red complex of bacteria post-treatment, similar to other studies that also reported decreases in proportions of this complex of bacteria (Cugini *et al.*, 2000; Haffajee *et al.*, 1997b; Socransky *et al.*, 2004). The study by Carvalho *et al.*, (2005) also found decreases in the red complex bacteria in the group that received SRD and placebo as well as the group that received SRD with weekly supragingival plaque removal for three months and a placebo (Carvalho *et al.*, 2005). Interestingly, no changes were observed in percentages of the red complex post-SRD for any of the levels analysed. Mean rank data showed no statistical differences between the test and control groups post-SRD.

4.4.5.1.2 Changes in levels of red complex bacteria at twelve months

At twelve months, further reductions were seen in percentages of the red complex bacteria in both groups. The study by Haffajee *et al.*, (2008) also reported reductions of the red complex bacteria following SRD which was maintained at twelve months following therapy (Haffajee *et al.*, 2008a).

From the mean rank data, the control group had higher numbers of the red complex bacteria as compared to the test group. However, no statistical significance was observed between the two groups at twelve months.

More reduction in percentages in the test group were noted for level 2 or “*increasing bacterial load*” and level 3 or “*high bacterial load*” as compared to the control group. There was also a higher percentage of the red complex bacteria recorded at levels 0 or “*bacterial load under cut off*” in the test group. This is suggestive that the SRD-with perioscope was more successful in reducing the number of pathogens in the red complex, at twelve months as compared to SRD-only. The SRD-with perioscope also reported a significantly lower mean PPD, mean BOP and mean PI as compared to the control group at twelve months. The study by Haffajee *et al.*, (1997) using the checkerboard DNA-DNA hybridization techniques to analyse microbiota, found that SRD did affect most bacteria; however, three pathogens namely *P. gingivalis*, *B. forsythus*, *T. denticola* decreased significantly after SRD. However, these bacteria and other bacteria were not completely eliminated and were still detectable post-therapy. By three months, most of the sites were recolonised and returned to pre-therapy levels, similar to what was described by Slots *et al.*, in 1979 (Haffajee *et al.*, 1997b; Slots, 1979). For the current study, the red complex bacteria reduced in both groups, percentages did not reach pre-SRD percentages, however, the red complex bacteria were not completely eliminated in both groups.

4.4.5.2 Effect of SRD-only and SRD-with perioscope on the orange complex

The orange complex of bacteria are thought to be major causative agents of periodontal disease with bacteria in this complex having moderate to strong pathogenicity (Socransky *et al.*, 1998). Pre-SRD, higher numbers of the orange

complex of bacteria were detected in the test group with higher percentages especially for level 3 or “*high bacterial loads*” (21.1%) as compared to the control group (5.3%).

4.4.5.2.1 Changes in levels of the orange complex post-SRD

Post-SRD saw reductions in both groups with the control group recording lower numbers of the orange complex bacteria than the test group. However, minimal differences were noted between groups with no statistical significance found between the two groups. Reductions in the orange complex bacteria for the group that received SRD plus weekly professional supragingival plaque removal for three months was also reported in the study by Carvalho *et al.*, (2005) (Carvalho *et al.*, 2005).

4.4.5.2.2 Changes in levels of the orange complex at twelve months

At twelve months, percentages of the orange complex decreased in both groups with the test group doing better in reducing the numbers of the orange complex bacteria as compared to the control group. This was evident in the decrease of percentage of level 3 or “*high bacterial loads*” to 0% at twelve months. No significant differences were reported between groups at twelve months.

The red complex is rarely found in the absence of the orange complex as demonstrated by Socransky *et al.*, (1998) and with “increasing colonization of the orange complex more sites were colonised by increasing numbers of the red complex” (Socransky *et al.*, 1998). Even though no statistical significance was reached, the test group had more reductions in both the red and orange complex of bacteria at twelve months as compared to the control group. Socransky *et al.*, (1998) also reported a significant association with the red and orange complex and increasing pocket depths and BOP (Socransky *et al.*, 1998). One could infer that the lower numbers of the both the red and orange complex in the SRD-with perioscope group could be related to the significantly lower mean PPDs and lower BOP at twelve months as compared to the SRD-only group.

4.4.5.3 Effects of SRD-only and SRD with perioscope on the orange-associated complex

The orange-associated bacteria appear immediately after the early-colonizers. Bacteria in this complex have low pathogenicity except for *E. nodatum* (Socransky *et al.*, 1998). The orange-associated complex of bacteria were reported in higher numbers in the control group as compared to the test group pre-SRD as reflected in the higher mean ranks and higher percentages. However, no statistically significant differences were found between the groups.

4.4.5.3.1 Changes in levels of orange associated complex post-SRD

Post-SRD resulted in reductions of the orange-associated complex of bacteria in both groups with reductions in percentages of level 3 or “*high bacterial loads*”. Both groups had increases in percentages of level 0 or “*bacterial load under cut off*”. From the mean ranks and percentages at each level for the orange-associated complex of bacteria, the test group was better in reducing the numbers of this complex at three months as compared to the control group. The study by Colombo *et al.*, (2005) also found that both *C. rectus* and *E. nodatum* (orange-associated complex bacteria) reduced in frequency and counts post-therapy (Colombo *et al.*, 2005).

4.4.5.3.2 Changes in levels of the orange-associated complex at twelve months

At twelve months, the levels of the orange-associated complex remained below pre-SRD levels. Both groups had high percentages of this complex at level 0 and level 1. The percentages of the orange-associated complex at level 2 or “*increased bacterial loads*” and level 3 or “*high bacterial loads*” were lower in the control group than the test group with the SRD-only group having less of the orange-associated complex bacteria as compared to the test group at twelve months. No statistically significant differences were found between the groups.

4.4.5.4 Effects of SRD-only and SRD-with perioscope on the green complex

The bacteria of the green complex are considered early colonisers and are facultative anaerobic species with moderate pathogenicity whose concentration increases with probing depths (Socransky *et al.*, 1998). The test group had higher numbers of the green complex bacteria as compared to the control group pre-SRD.

4.4.5.4.1 Changes in levels of the green complex post-SRD

Post-SRD resulted in reductions of the green complex of bacteria in both groups. Decreases in level 2 and 3 were observed in the control group, with the test group having decreases at level 3 only.

The green complex bacteria were detected at high numbers in the test group as compared to the control group post-SRD, with no statistical significance reported between the groups.

4.4.5.4.2 Changes in levels of the green complex at twelve months

At the twelve months analysis, the control group accomplished more reduction in numbers of bacteria in the green complex as observed by the mean rank data. Percentages at level 3 or “*high bacterial loads*” decreased from 21.1% post-SRD to 0% in the control group as compared to 31.6% in the test group. Interestingly 21.1% of the green complex was recorded at twelve months for the control group at level 4 or “*extremely high bacterial loads*” and 0% for the test group at this level. This could be related to the pooling of samples. One can conclude that the SRD-only was more effective in reducing these early colonizers as compared to the SRD-with perioscope at all time points.

4.5 Final Comments

In conclusion, even though there were no significant differences found between the control and test groups with regards to bacterial colonization of the eleven pathogens and the complexes of bacteria, the following information can be extrapolated from the analysis:

- Irrespective of treatment modality used, both treatments resulted in reduction of most of the bacteria with only slight differences observed between the groups.
- *A. actinomycetemcomitans*, *C. rectus* and *Capnocytophaga sp.* reduced post-SRD in both groups, with some recolonization occurring at twelve months in both groups. Bacterial recolonization is a common occurrence and can occur quite quickly after treatment (Cugini *et al.*, 2000; Haffajee *et al.*, 1997b; Harper *et al.*, 1987; Shiloah *et al.*, 1994).
- Lowering levels of periodontal pathogens to below pre-treatment levels is one of the goals of periodontal therapy (Teles *et al.*, 2006). Both treatment modalities resulted in reduction of *P. gingivalis*, *T. denticola*, *T. forsythia*, *P. intermedia*, *P. micros*, *E. nodatum* and *E. corrodens* post-SRD and at twelve months with pathogen levels not returning to pre-SRD levels.
- *F. nucleatum* increased post-SRD in both groups and then decreased at twelve months. However, *F. nucleatum* are considered commensal inhabitants and are difficult to reduce or eliminate by SRD (Feres *et al.*, 2001; Page, 1995).
- Both test and control groups had persistent deep pockets at the time of microbiological testing. Pooling of samples included deep pockets which could have in turn affected the percentages of pathogens at each level.
- Even though the percentage of *A. actinomycetemcomitans* at level 4 or “extremely high load” at twelve months was low (10.53% in the control group and 15.79% in the test group), this could be a result of the samples taken from persistent deep pockets. Similarly, the small percentage of *E. nodatum* detected at level 3 or “high bacterial loads” could be linked to residual deep PPDs.
- The persistence of *P. gingivalis* at level 2 or “increasing bacterial load”, *T. forsythia* at level 3 or “high bacterial load and level 4 or “extremely high bacterial load” and *T. denticola* and *T. denticola* at level 2 or “increasing bacterial load” and level 3 or “high bacterial load” may be suggestive that active, non-

responding sites are still present. The co-colonization and interaction of these three pathogens needs to be considered.

- The small percentage of *P. intermedia* detected at level 3 or “*high bacterial load*” could be related to disease activity.
- The detection of *P. micros* at level 2 or “*increasing bacterial load*” and level 3 or “*high bacterial load*” more especially in the SRD-only group could suggest active, progressing sites.
- The detection of *C. rectus* at level 2 or “*increasing bacterial load*” and level 3 or “*high bacterial load*” needs to be monitored because of the link of this pathogen with disease recurrence.
- SRD-with perioscope resulted in no changes in numbers of pathogens grouped in the red complex post-SRD, however at twelve months, more reduction occurred in this group as compared to the SRD-only group.
- Both the red and orange complexes were detected at lower percentages in the test group at twelve months. This could be related to the significantly lower mean PPD and BOP in this group as compared to the control group.
- The early colonizers (the green complex) and orange associated complex which appears immediately after the early colonizers had more improvement in bacterial numbers in the control group.

Decreases in numbers of pathogens varied among groups, making it difficult to make clear assumptions that one group performed better than the other with reduction in numbers of periodontal pathogens. However, one can infer that the control group had more reductions in pathogen numbers at three months than the test group. At twelve months, the test group had more reductions in pathogen numbers of the red and orange complex bacteria which are considered primary periodontal pathogens.

Healing can take 9-12months for maximum resolution following therapy (Badersten *et al.*, 1981; Badersten *et al.*, 1984a; Cobb, 2002; Cugini *et al.*, 2000; Kaldahl *et al.*, 1988; Morrison *et al.*, 1980). The technique using the perioscope for SRD involves insertion of the endoscope subgingivally, identification of the subgingival deposits and then removal of the deposits. The technique is considered less invasive as once calculus is identified, the instruments are placed directly on the deposits and removed with no

intentional removal of granulation tissue and little interference to the soft tissue. Whether more decreases in percentages of the bacteria in the test group at twelve months as compared to the control group is related to technique used and potentially longer healing is hard to ascertain. This longer healing time was also realised with the use of the perioscope when compared to SRD-only where significant reduction of mean pocket depths, bleeding upon probing, PPDs 7-9 mm occurred at twelve months as compared to post-SRD.

The role of SPT is reducing and maintaining bacterial loads below detectable levels also needs to be considered. Many studies have supported the important role of SPT in reducing numbers of pathogens (Cugini *et al.*, 2000; Haffajee *et al.*, 1997b; Rosling *et al.*, 2001) with reducing the levels of pathogens to manageable levels being the goal of treatment (Kamma *et al.*, 2003). For this study, participants in both groups were seen every three months for SPT. Some of the pathogens were kept at manageable levels with low percentages recorded for “high” and “extremely high” levels of bacteria including, *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia* and *E. nodatum*. However, *T. denticola*, *T. forsythia*, *Fusobacterium nucleatum*, *C. rectus*, *E. corrodens*, *P. micros* and *Capnocytophaga sp.* were still found at levels above threshold following treatment and SPT which could be related to the pooling of samples including persistent deep pockets. When examining the complexes of bacteria, reduction in numbers of the red and orange complex were seen in both groups with the test group having lower numbers at twelve months. The red complex was found less frequently and in low numbers in healthy and well-maintained patients in the study by Haffajee *et al.*, (1998) (Haffajee *et al.*, 1998). SRD followed by three monthly SPT was found to reduce the prevalence of *B. forsythus*, *P. gingivalis* and *T. denticola* up to 6 months and bacterial levels remained low at 9 and 12 months (Cugini *et al.*, 2000). In five-year follow-up study by Kamma *et al.*, 2003, SPT was found effective in maintaining periodontal stability; however, there were a small number of sites that exhibited periodontal progression. High bacterial counts of *P. gingivalis* and *T. denticola* were found in these sites. Other factors such as smoking, stress and number of acute episodes were also considered contributing factors (Kamma *et al.*, 2003). In the current study, *T. denticola* and to a lesser extent *T. forsythia* were still reported to have high numbers following SPT suggesting that participants need to be closely monitored for progressing sites.

One also needs to consider the methodology used for microbiological analysis. For this clinical study, the Micro-IDent plus test was used. This test uses DNA strip technology to detect bacteria. The test involved supplying pooled samples of the five areas sampled at each time point. At baseline, plaque samples were taken of five of the deepest sites and the exact same sites were sampled at three and twelve months. Results provided a quantitative determination of the bacterial load of the pooled sample provided. It is evident from the data that some sites still had deep pockets post-SRD and at twelve months with the analyses of pooled samples impacting on the microbiological results for participants. PPDs at baseline ranged from 5.4 mm to 13 mm; 2.80 mm to 9.4 mm at three months and 2.6 mm to 9.4 mm at twelve months for both groups. Of the five pockets assessed there could have been one pocket that harboured high numbers of pathogens which in turn could have affected the results obtained. We are aware that deep pockets harbour more bacteria, and this could have influenced the bacterial levels of the pathogens at the different time points. The assessment of individual sites would have been more beneficial, however the cost associated with analysing individual sites had to be considered for the current study.

The Micro-Ident plus test provides a semi-quantitative result. The methods used is based on the 16SrDNA where precise numbers of the bacteria is not possible due to the fact that each cell may contain a variable amount of this molecule (Morillo *et al.*, 2003). This is not an accurate representation of the actual quantity or numbers of bacteria, which could in turn affect the results obtained. In addition, the methodology used for statistical analysis involved ranking of the ordinal variables. The Mann Whitney *U* test provides information of the relative magnitudes of the observations in the two groups, making it difficult to determine the degree of difference between variables.

Another possible limitation is the methodology used when removing the plaque samples. For the current study, the area was dried but no removal of supragingival plaque was done prior to inserting the sterile paper point subgingivally. The instructions provided in the kit did not specify any removal of supragingival plaque prior to inserting the sterile point into the periodontal pocket and was therefore not implemented. However, Beikler *et al.*, (2006) found that providing a sample of both supra and subgingival plaque taken from deepest periodontal pocket provides the most reliable

result (Beikler *et al.*, 2006). Chaves *et al.*, (2000) wiped the area prior to obtaining plaque samples but there is no indication of whether removal of supragingival plaque samples occurred (Chaves *et al.*, 2000), similar to Colombo *et al.*, (2009) where subgingival plaque samples were obtained without removal of supragingival plaque (Colombo *et al.*, 2009). However, previous studies have involved the removal of supragingival plaque samples prior to obtaining a subgingival plaque sample (Booth *et al.*, 2004; Brochut *et al.*, 2005; Buchmann *et al.*, 2000; Byrne *et al.*, 2009; Charalampakis *et al.*, 2013; Corraini *et al.*, 2013; Cugini *et al.*, 2000; da Silva-Boghossian *et al.*, 2011; Dahlen *et al.*, 2006; Darby *et al.*, 2001; Doungudomdacha *et al.*, 2001; Dowsett *et al.*, 2002; Eick *et al.*, 2002; Eick *et al.*, 2011; Haffajee *et al.*, 1997b). Whether the microbiota in the supragingival plaque affected the numbers of pathogens detected is hard to ascertain for this study. However, supragingival contamination would have been minimal as paper points were inserted subgingivally.

The information extrapolated from the pooled analyses provided information or suggestions on recommended antibiotic therapy according to the DG Paro (German Society for Periodontology and the Association for the scientific promotion of medicine and dentistry with a focus on periodontics). These recommendations were not followed for this study as the aim of the study was to test the effect of SRD-only and the SRD-with the perioscope with no use of adjunctive antibiotics. There are numerous studies that have been conducted on the use of systemic antibiotic therapy in conjunction with non-surgical debridement. However, controversy exists with the benefits only noted in selected cases. Han *et al.*, (2012) conducted a double-masked placebo-controlled study to investigate the effect of SRD plus the use of azithromycin on patients with severe chronic generalised periodontitis. Both groups showed significant improvements in clinical parameters in deep and moderate pockets, however no additional benefit of using azithromycin over non-surgical periodontal treatment was found. Microbiological analysis showed that azithromycin did not provide any additional effect of periodontal pathogens. *F. nucleatum*, *P. gingivalis*, *P. intermedia*, and *T. forsythia* decreased in both groups, none were eradicated, and most bacteria tended to reappear after 6 months except for *P. intermedia* (Han *et al.*, 2012). Similarly, Fonseca *et al.*, (2015) found groups treated with azithromycin did not show statistically significant differences in pocket depths, reduction in percentage of periodontally diseased sites or lower microbial levels at 90 and 180 days after treatment (Fonseca

et al., 2015). However, Haffajee *et al.*, (2003) found that the greatest reduction of red and orange complex bacteria were in subjects who received SRD, plus systemically administered metronidazole and continued professional supragingival plaque removal (Haffajee *et al.*, 2003a). However, data did not include results beyond three months which can make it difficult to ascertain whether the effects of adjunctive therapies can be sustained over longer periods. *A. actinomycetemcomitans* which has been linked to a more aggressive form of periodontal disease can be difficult to eradicate non-surgically alone and some benefits of use of systemic antibiotics have been seen for patients with high levels of *A. actinomycetemcomitans* with deeper pockets (Mombelli *et al.*, 2000). The meta-analysis by Keestra *et al.*, (2015) included 45 clinical studies and compared the effectiveness of systemic antibiotics on clinical parameters and examined their long-term effects up to one year. This review found that SRD with systemic antibiotics did result in improvement clinical outcomes as compared to SRD alone. Metronidazole or metronidazole combined with amoxicillin was found to be more beneficial for moderate to deep pockets as compared to azithromycin, although the effects decreased over time (Keestra *et al.*, 2015). Bacterial resistance is a common occurrence especially with overuse of systemic antibiotics. This was observed in the study by Winkelhoff *et al.*, (2005) who reported a major susceptibility difference profile between periodontal pathogens isolated from patients with periodontitis in Spain and the Netherlands (van Winkelhoff *et al.*, 2005). The inclusion of systemic antibiotics as part of non-surgical periodontal therapy needs to be assessed on a case by case basis.

In conclusion, the following recommendations can be made for future studies:

- The Micro-IDent test by Hain Lifescience is a simple chairside test, however, the analysis of pooled samples and persistent deep pockets may have influenced levels and percentages of the pathogens analysed as reflected in the results. Analysis of individual sites is recommended which will allow correlation analysis with clinical parameters.
- Bacteria need to reach specific thresholds before they can cause disease. Sampling of individual sites and determination of these thresholds may have been more beneficial in assisting with predicting disease progression.

CHAPTER 5

RADIOGRAPHIC ANALYSIS

This chapter describes the results of the radiographic analysis. Standardised radiographs were taken at pre-treatment and at twelve months in both the test and control groups and compared using linear measurements.

The number of radiographs taken at pre-treatment and at twelve months as well as the number of radiographs that were eligible for assessment are included. Sites displaying vertical and horizontal bone loss were calculated for each group.

The change in RBLs between pre-treatment radiographs and radiographs taken at twelve months were analysed. Teeth included in the analysis were central and lateral incisors, canines, 1st and 2nd premolars, 1st and 2nd molars. Teeth were also categorised as single-rooted (central and lateral incisors, canines and 2nd premolar) and multi-rooted teeth (1st premolar and 1st and 2nd molars). Changes in mean RBLs for single and multi-rooted teeth is also described. Independent sample T-tests were used to test differences between mean RBL between the test and control groups. Confidence intervals are reported and $p < 0.05$ was considered statistically significant. Changes in RBL were categorised into 0.5 mm increments and the frequency and range are described.

The percentage of sites with radiographic bone loss, bone gain and no changes in RBLs in sites displaying vertical and horizontal bone loss were calculated for both study groups.

5.1 Radiographic Analyses

All thirty-eight patients were included in the radiographic analysis. Table 5.1 displays information on the number of radiographs taken pre-SRD, at twelve months and the number of radiographs eligible for measurement and assessment. Each pre-treatment radiograph had to be compared to radiographs taken at twelve months and compared for accuracy before analysis took place.

Not all radiographs taken at baseline could be repeated at twelve months. Exact replication of placement of the positioning stent was not possible in some cases due

to patients' strong gag reflex and the inability to tolerate the holders together with the positioning stent. There were a few positioning stents that were damaged which affected the bite registration and therefore these could not be re-used.

When it came to analysis of radiographs, radiographs that were not the exact replica of radiographs taken pre-SRD were removed from the analysis. Radiographs taken pre-SRD were compared for accuracy with radiographs taken at twelve months. The placement of the positioning stent was examined with reference to how the stent fitted onto the teeth. The teeth and the sites present on the pre-treatment and twelve-month radiographs were carefully examined by placing radiographs side-by side. If there was a discrepancy with the teeth or sites present between radiographs, this indicated incorrect placement of the positioning stent and these radiographs were removed from the analysis. Radiographs were also removed if there was overlapping of the images due to incorrect angulation as well as blurring of images due to movement of the patient or radiograph during exposure. Even though the position of the bite stent was correct, slight movement affected the exact replication of the radiograph.

In the control group only 66 of the 82 radiographs taken could be assessed. Two patients could not tolerate the holders with the positioning stent due to a strong gag reflex. This resulted in four of the posterior radiographs unable to be taken at the re-evaluation appointment. Two radiographs had damage to the positioning stent and was unable to be re-used. Ten radiographs were excluded from the analysis due to radiographs not being the exact replica of radiographs taken pre-SRD.

In the test group, 71 of the 78 radiographs taken were eligible for assessment. One positioning stent could not be re-used as the stent was damaged. Six radiographs were excluded from the analysis as radiographs were not the exact replication of the radiographs taken at baseline.

In total, the control group had 396 sites suitable for analysis, compared to 420 sites in the test group. (Table 5.1) Of the 396 sites in the control group, 151 sites displayed horizontal bone loss and 245 sites displayed vertical or angular bone loss. In the test group, 154 of the sites had horizontal bone loss and 266 sites had vertical/angular bone loss. Percentage of sites with vertical and horizontal bone loss for single and multi-rooted teeth are included. (Table 5.2)

Table 5. 1 Total number of radiographs taken at baseline (pre-SRD) and twelve months and total number of sites for control and test groups

	Control (n=19)	Test (n=19)
No of radiographs taken Pre-SRD	89	79
No of radiographs taken Post-SRD	82	78
Total no of radiographs assessed at twelve-months	66	71
Total no of sites	396	420

Table 5. 2 Number and percentage of surfaces with horizontal and vertical/angular bone loss in the control and test group for all teeth and single and multi-rooted teeth

	Control (n=19)	Test (n=19)
Total sites	396	420
Number of sites with horizontal bone loss		
	151 (38.1%)	154 (36.7%)
Number of sites with vertical bone loss		
	245 (61.9%)	266 (63.3%)
Single rooted teeth		
Total sites	231	204
Number of sites with horizontal bone loss		
	82 (35.5%)	85 (41.7%)
Number of sites with vertical bone loss		
	149 (64.5%)	119 (58.3%)
Multi-rooted teeth		
Total sites	165	216
Number of sites with horizontal bone loss		
	69 (41.8%)	69 (31.9%)
Number of sites with vertical bone loss		
	96 (58.2%)	147 (68.1%)

5.1.1 Effect of SRD-alone and SRD-with the perioscope on radiographic bone levels

When comparing RBL pre-treatment and at twelve months, the mean change in RBL was higher in the test group (0.69 ± 0.3 mm) as compared to the control group (0.49 ± 0.2 mm) demonstrating that there was more bone gain in the test group as compared to the control group. This difference in mean RBL was statistically significant

(p=0.03) (Table 5.3). Figure 5.1 shows the mean change in RBL and confidence intervals for both the test and control groups.

The minimum and maximum change and range for RBL is presented in Table 5.3. Figure 5.2 displays the range and frequency of RBL for both the control and test groups. From this figure it is evident that at twelve months the test group had improved RBLs with a higher frequency of RBLs between 0.5 mm to 1.0 mm and 1.0 to 1.5 mm as compared to the control group. (Figure 5.2)

Table 5. 3 Statistical analyses of mean change (mm) in radiographic bone levels at baseline (pre-SRD) and at twelve months between control and test groups

Control (n=19) SRD - only					Test (n=19) SRD- with perioscope					Independent sample t-test summary statistics	
Mean, std deviation	Std error of mean	Min	Max	Range	Mean, std deviation	Std error of mean	Min	Max	Range	95% CI	p- value
0.49 ±0.2	0.05	0.06	0.97	0.92	0.69 ±0.3	0.08	0.10	1.48	1.38	-0.4; -0.0	0.03

Figure 5. 1 Mean change in RBL (mm) and confidence levels for both control and test groups (pre-SRD and twelve months)

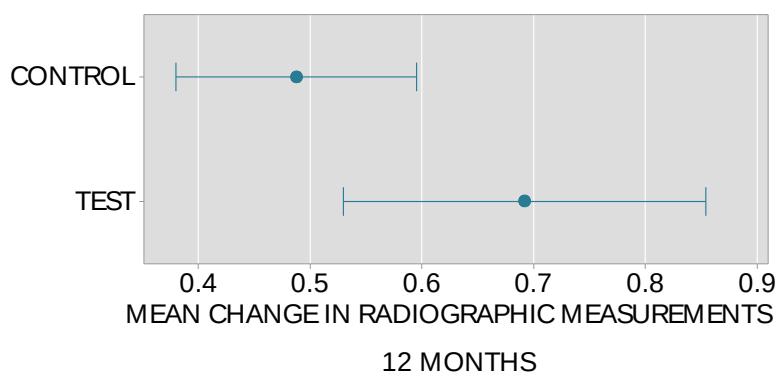
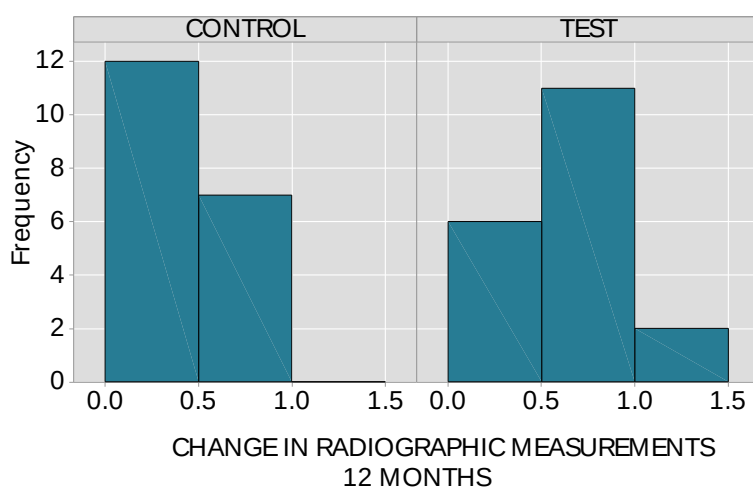


Figure 5. 2 Histogram showing range and frequency for change in RBL for control and test groups at twelve months



5.1.2 Effect of SRD-alone and SRD-with perioscope on radiographic bone levels on single-rooted and multi-rooted teeth

Teeth were divided into single and multi-rooted teeth and the analyses of RBLs are presented in Table 5.4

5.1.2.1 Single-Rooted Teeth

The mean change in RBL for single-rooted teeth was only marginally higher in the test group (0.52 ± 0.28 mm) as compared to the control group (0.49 ± 0.32 mm). This difference in mean RBL for single-rooted teeth between the test and control group was not statistically significant ($p=0.8$) (Table 5.4). Figure 5.3 displays the mean change in RBL (mm) and confidence intervals for the control and test groups for single-rooted teeth.

The minimum, maximum and range in millimetres is described in Table 5.4. The histogram in Figure 5.4 depicts the frequency of RBL at 0.5 mm intervals for single-rooted teeth for both treatment groups. From this graph, there is a trend showing that the test group had slightly more changes in RBL within the 0.5 mm to 1.0 mm and 1.0 to 1.5 mm.

Table 5. 4 Statistical Analyses of mean change (mm) in radiographic bone levels at baseline (pre-SRD) and at twelve months for control and test groups for single and multi-rooted teeth

Control (n=19) SRD - only					Test (n=19) SRD- with perioscope					Independent sample t-test summary statistics	
Mean, std deviation	Std error of mean	Min	Max	Range	Mean, std deviation	Std error of mean	Min	Max	Range	95% CI	p- value
Single rooted teeth											
0.49 ±0.32	0.1	0.03	1.43	1.40	0.52 ±0.28	0.1	0.08	1.13	1.05	-0.2; 0.2	0.8
Multi-rooted teeth											
0.46 ±0.36	0.1	0.01	1.11	1.10	0.83 ±0.45	0.1	0.18	1.93	1.75	-0.6; -0.1	0.00

Figure 5. 3 Mean change in RBL (mm) and confidence intervals for single- rooted teeth for control and test groups

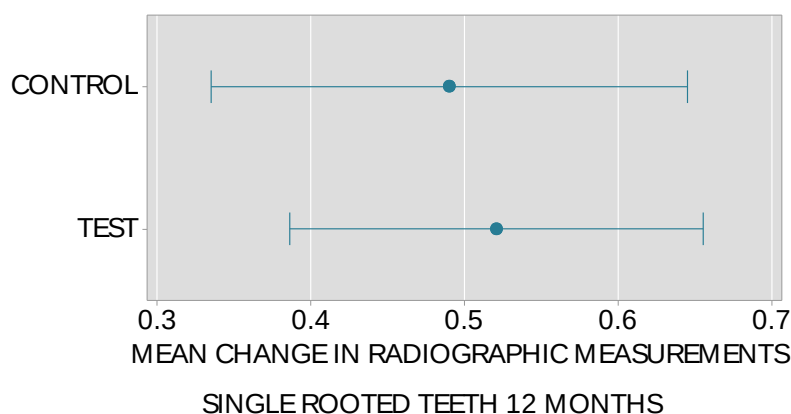
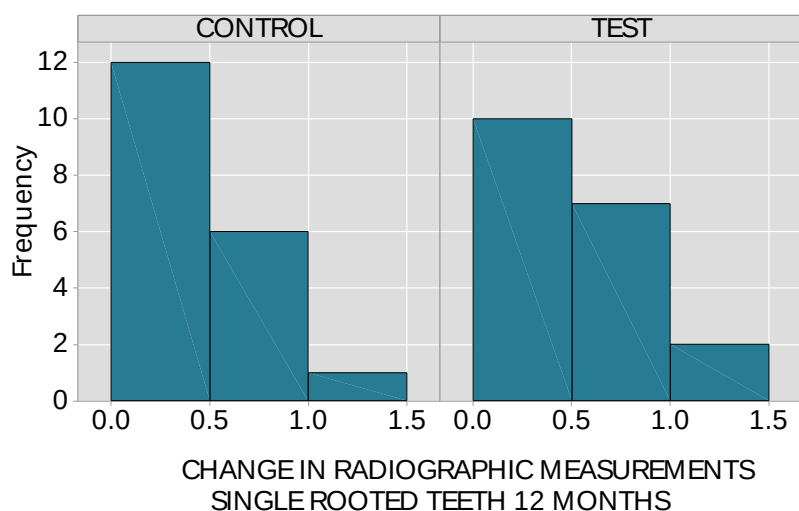


Figure 5. 4 Histogram showing range and frequency of change in RBL for single-rooted teeth for control and test groups at twelve months



5.1.2.2 Multi-Rooted Teeth

For multi-rooted teeth, a higher mean change in RBL were reported for the SRD-with perioscope group (0.83 ± 0.45 mm) as compared to the SRD-only group (0.46 ± 0.36 mm). This difference in mean change in RBL was statistically significant ($p=0.00$). (Table 5.4) Figure 5.5 shows the mean change in RBL and confidence intervals for multi-rooted teeth. The minimum, maximum and range in millimetres is described in Table 5.4 for multi-rooted teeth. From the histogram, it is evident that the test group had a higher frequency in RBL from 0.5 mm to 1.0 mm than the control group at twelve months. In addition, the control group did not have any changes in RBL from 1.5 mm to 2.0 mm; whereas the test group did record change in RBL for this range (Figure 5.6)

Figure 5. 5 Mean change in RBL (mm) and confidence intervals for multi-rooted teeth for control and test groups

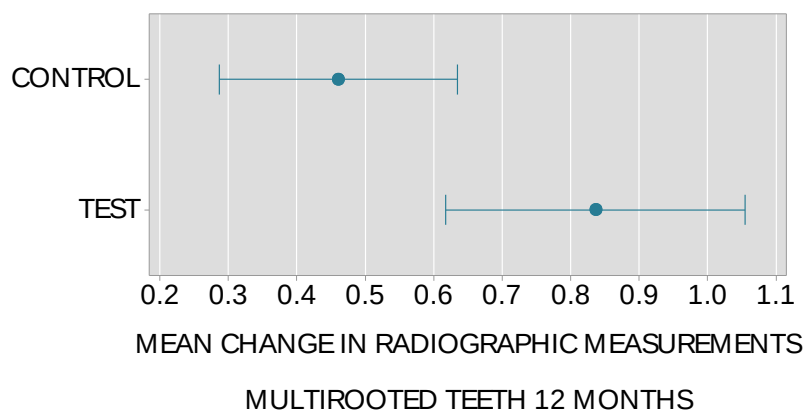
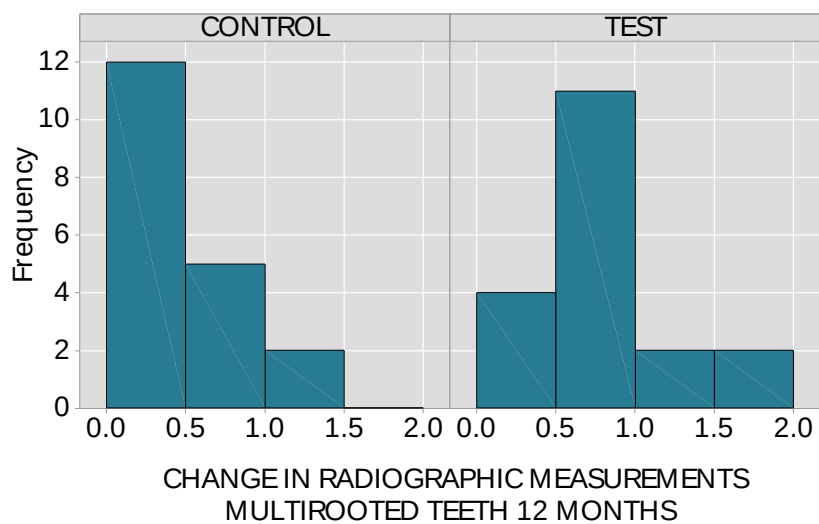


Figure 5. 6 Histogram showing range and frequency of change in RBL for multi-rooted teeth for control and test groups at twelve months



5.2 Comparison of radiographic bone loss, bone gain and no change in bone levels between test and control groups

The total number of sites displaying horizontal and vertical/angular bone loss is described in Table 5.2. There were minimum differences between the test and control groups with surfaces with horizontal and vertical bone loss for all teeth and single and multi-rooted teeth.

Table 5.5 includes the percentage of sites with bone loss, bone gain or no changes in bone height in the areas displaying vertical/angular bone loss and horizontal bone loss for all teeth and single and multi-rooted teeth.

In sites with vertical bone loss, the test group had a higher percentage of sites with bone gain (89.1%) as compared to the control group (82%). Fewer sites displayed bone loss in the test group (3.8%) as compared to the control group (5.7%). The test group had a lower percentage of sites with no change to bone levels (7.1%) as compared to the control group (12.3%). When comparing single-rooted teeth and multi-rooted teeth, the test group also had a higher percentage of sites with bone gain as compared to the control group. No change in RBL were observed in majority of sites displaying horizontal bone loss (92.1% in the control group and 96.1% in the test group). Horizontal bone loss was recorded in only few sites in the control (7.9%) and test groups (3.9%).

Table 5. 5 Number and percentage of surfaces with vertical/angular bone loss and horizontal bone loss displaying radiographic bone gain, bone loss and no change in radiographic bone levels for all teeth and single and multi-rooted teeth

	Control (n=19)	Test (n=19)
VERTICAL BONE LOSS		
All teeth		
Total sites	245	266
Radiographic bone gain (%)	201 (82%)	237 (89.1%)
Radiographic bone loss (%)	14 (5.7%)	10 (3.8%)
No change in RBL (%)	30 (12.3%)	19 (7.1%)
Single rooted teeth		
Radiographic bone gain (%)	124 (83.2%)	106 (89.1%)
Radiographic bone loss (%)	7 (4.7%)	5 (4.2%)
No change in RBL (%)	18 (12.1%)	8 (6.7%)
Multi-rooted teeth		
Radiographic bone gain (%)	77 (80.2%)	131 (89.1%)
Radiographic bone loss (%)	7 (7.3%)	5 (3.4%)
No change in RBL (%)	12 (12.5%)	8 (6.7%)
HORIZONTAL BONE LOSS		
All teeth		
Total sites	151	154
Radiographic bone gain (%)	0 (0%)	0 (0%)
Radiographic bone loss (%)	12 (7.9%)	6 (3.9%)
No change in RBL (%)	139 (92.1%)	148 (96.1%)
Single rooted teeth		
Total sites	82	85
Radiographic bone gain (%)	0 (0%)	0 (0%)
Radiographic bone loss (%)	6 (7.3%)	3 (3.5%)
No change in RBL (%)	76 (92.7%)	82 (96.5%)
Multi-rooted teeth		
Total sites	69	69
Radiographic bone gain (%)	0 (0%)	0 (0%)
Radiographic bone loss (%)	6 (8.7%)	3 (4.3%)
No change in RBL (%)	63 (91.3%)	66 (95.7%)

5.3 Discussion and Summary of Findings

Bone loss and osseous defects are a consequence of periodontal disease. Radiographs allow for examination of the crestal bone, crown-root ratios, horizontal and vertical bony defects, furcation areas and the morphology of the bone (Zaki *et al.*, 2015). Radiographic changes in patients with periodontal disease initially has a “fuzzy” appearance with discontinuity of the lamina dura on the mesial and distal surfaces of the interdental septum. A wedge-shaped radiolucency occurs in the interdental septa and with continued destruction, it will extend across the interdental septa and result in reduction of height of the septum, resulting in a bony defect (Manson *et al.*, 1974).

Radiographs are valuable diagnostics aids and can provide important information about disease resolution and progression. However, comparative analysis of both clinical and radiographic data is important in diagnosis of periodontal disease. Alveolar bone loss can be slow and continuous or intermittent with bone loss either horizontal or vertical/angular (Greenstein *et al.*, 2009).

Osseous changes following SRD have been reported in studies with many different methods used to assess and analyse changes in radiographs following therapy. Some studies used linear measurements where radiographic bone height are measured from the CEJ to the alveolar crest parallel to the long axis of the tooth (Nibali *et al.*, 2015b; Nibali *et al.*, 2011) and while some used digital subtraction methods enabling detection in changes in bone volume (Hwang *et al.*, 2008). The current study evaluated the effects on SRD-only and SRD-with perioscope on RBLs using linear measurements.

Not all studies revealed changes in RBLs following non-surgical therapy. Some reported only minimal change in alveolar bone levels (Preshaw *et al.*, 2005; Rosling *et al.*, 2001). Radiographic changes in infrabony defects were compared 12 months after completion of three treatment modalities including the modified Widman flap, reverse bevel surgery and non-surgical periodontal therapy. A full series of radiographs were taken pre-treatment using the bisecting angle technique. In this study, RBLs remained unchanged following non-surgical therapy. Standardised radiographs were not used which potentially could have resulted in errors when making comparisons between pre and post-treatment radiographs (Isidor *et al.*, 1986).

Nonetheless, alveolar bone changes following periodontal treatment has been reported in some studies. In 1985, Rethman *et al.* found increases in alveolar bone density in treated quadrants for the Rhesus monkey (Rethman *et al.*, 1985). Data presented on 46 sites analysed with digital subtraction radiography reported a statistically significant difference in bone density changes between treated and control sites in the Rosling *et al.*, study in 1998 (Rosling *et al.*, 1983). Likewise alveolar bone changes correlated with PALs in 20 defects, 16 weeks after therapy in patients with juvenile periodontitis (Christersson *et al.*, 1985). Changes in bony defects and radiographic bone fill were also described in radiographs taken one year after therapy in a clinical case report on patients with generalised aggressive periodontitis (Jung *et al.*, 2010).

For the current study, standardised radiographs were taken of the teeth with the deepest pocketing and in sites displaying vertical/angular bone loss for each patient pre-SRD and compared to radiographs taken at twelve months. Digital radiographic imaging techniques was utilized which not only minimized the exposure to patients but also provided high resolution, better quality images which in turn improved diagnostic interpretation (Woolhiser *et al.*, 2005). Standardising parameters such as the radiographic focal sensor distance, sensor position and x-ray beam angulation with use of paralleling devices and positioning stents produced a more reproducible repositioning of the radiographic device and improved the precision of comparative measurements.

Bone levels were measured using linear measurements from the CEJ to the bony crest. This method has previously been used in other epidemiological studies (Machtei *et al.*, 1998; Nibali *et al.*, 2015b; Nibali *et al.*, 2011; Papapanou *et al.*, 1989). Changes in mean RBL were observed for both groups at twelve months, with the mean change in RBL significantly higher in the SRD-with perioscope group as compared to the SRD-only group. There are no studies to date that examined the use of the perioscope and its effects on RBLs. A large number of case series showing changes between pre-treatment and post-perioscopy radiographs were reported by Harrel *et al.*, (2015) (Harrel *et al.*, 2015). Post-treatment radiographs taken varied from 15 months to 8 years. There appeared to be radiographic changes post-perioscopy in these case series, however there was no suggestion that radiographs taken were standardised.

Variations in appearances on radiographs can be a result of changes in angulations of the x-ray beam (Bender, 1997), making it difficult to accurately compare changes in RBL for the pre- and post-treatment radiographs included in these case series.

The findings of the current study are comparable to past and more recent studies comparing mean RBL post-therapy. The mean change in RBL in the one year f/up in the study by Machtei *et al.*, (1997) was 0.16 ± 0.2 mm (Machtei *et al.*, 1997b). The mean change in RBL is lower as compared to the current study for both the test (0.69 ± 0.3 mm) and control groups (0.49 ± 0.2 mm). However, measurement errors were likely in the Machtei *et al.*, (1997) study as the radiographs were not standardised.

Findings of more recent studies by Nibali *et al.*, (2011, 2015) can be compared to the current study. Even though these studies were retrospective in nature, study protocols were similar to those followed in the current study. Infrabony defects were assessed following non-surgical periodontal therapy using clinical and radiographic measurements in the study by Nibali *et al.*, (2011). Patients were placed on a three-monthly maintenance programme and were re-evaluated at 12 and 18 months. This study found that the horizontal suprabony component of the defect stayed the same whereas changes were noted in the average vertical defect angle from 3.77 mm and 37.41° at baseline to 3.08 mm and 44.11° , respectively, at re-evaluation ($p < 0.001$) (approximately 0.69 mm gain). Some sites showed reduction of the defect depth measurement, while some sites showed that the infrabony defect had completely resolved and in a few cases complete bone fill was noted. Although the radiographs taken were not standardised, the authors concluded that following non-surgical periodontal therapy radiographic bone fill in infrabony defects is possible (Nibali *et al.*, 2011). The more recent study by Nibali *et al.*, (2015) assessed intrabony and infrabony defects following minimally invasive non-surgical periodontal therapy (MINST) which included extensive subgingival debridement using magnification lenses, microscopes and small instruments to minimize tissue trauma. The findings of this study also reported a 0.7 mm gain in the infrabony defect similar to the previous study by the same author (Nibali *et al.*, 2011). For the current study, the mean change in RBL in the SRD-with perioscope group was also reported as 0.69 ± 0.3 mm with the test group having a statistically significant mean change in RBL at twelve months ($p < 0.05$) as compared to the control group. The current study methods for analysing bone levels

were similar to the Nibali *et al.*, (2011 and 2015) studies in that measurements were made from the CEJ to the bony crest for each site and compared; however, the defect depth was not calculated for the present study.

Studies have utilised methods to standardise radiographs and have reported on percentage of sites with bone gain, bone loss or no changes in RBL following therapy. Percentages of sites with bone gain, bone loss and no change in RBL were computed for the current study in areas displaying vertical and horizontal bone loss and comparisons made between the two treatment groups. Customised bite blocks were used by Schmidt *et al.*, (1988) to standardise radiographs at pre-treatment. In their study, periodontal treatment included both surgical and non-surgical periodontal therapy. Radiographs were repeated at three to fourteen months post-treatment. Eighty-nine percent of the paired radiographs were able to be subtracted and analysed. The authors reported that most areas (67%) showed no changes in bone structures following periodontal treatment. Of the twenty three sites that had debridement only and no surgery, fourteen sites (61%) had bone gain and nine sites (39%) had bone loss (Schmidt *et al.*, 1988). Varying percentages of bone loss were reported in other studies. The mean alveolar bone loss in the cross sectional investigation by Fukuda *et al.*, (2008) was $20.60 \pm 12.12\%$ (Fukuda *et al.*, 2008), 12.9% in the study by Machtei *et al.* in 1997 (Machtei *et al.*, 1997b) and $18.12 \pm 1.88\%$ in the study by Machtei *et al.*, in 1998 (Machtei *et al.*, 1998). The percentage of sites with bone gain was $16.20 \pm 1.68\%$ in the same study (Machtei *et al.*, 1998). When comparing to the current study, bone gain in sites displaying vertical/angular bone loss were recorded at higher percentages with 82% of sites in the SRD-only group recording bone gain and 89.1% of sites in the SRD-with perioscope group. Bone loss was also recorded at lower percentages, 5.7% in the control group and 3.8% in the test group. Only 12.3% of sites in the control group had no change in RBL, while 7.1% of sites in the test group reported no changes in RBL at twelve months. However, one has to take into consideration that linear measurements can result in measurement errors, with only slight increases in measurements being computed as bone gain. Majority of sites displaying horizontal bone loss had no changes at twelve months (92.1% in the control group and 96.1% in the test group). There were few sites in the both the test (3.9%) and control group (7.9%) where horizontal bone loss was recorded.

Even though digital subtraction radiography provides information on changes in bone density and provides information on changes in gray levels, increases in radiographic density can infer alveolar bone gain. Comparison between findings of studies using digital subtraction radiography and the current study have been included. Radiographic density of coronal, middle and apical portions of defects were analysed using digital subtraction radiography following periodontal therapy in the study by Hwang *et al.*, (2008). Included in this study were thirteen subjects with a total of 39 sites with >3 mm of vertical bone loss. SRD was done on all participants and radiographic examination included pre-treatment and post-treatment (six months after SRD) radiographs. It was reported that 101 of 111 (83.3%) test regions recorded an increase in radiographic density with probing depths >5 mm showing a significant increase in bone density as compared to pocket depths <5 mm (Hwang *et al.*, 2008). Similarly, radiographic bone density was measured on interproximal sites with PPDs >5 mm in the study by Dubrez *et al.*, in 1990. A similar treatment protocol was followed with subgingival instrumentation done on the ten patients and radiographs were taken pre-treatment, immediately after treatment and then at two, six and twelve months. Radiographs were standardised using paralleling devices and impression material. Statistical significant increases were seen for both superficial and deep densities of interproximal bone at six months and one year after non-surgical periodontal therapy with the superficial bone density being 13% higher at six months and 16% higher at one year following treatment as compared to radiographs taken immediately after treatment (Dubrez *et al.*, 1990). The increases in bone densities in these studies is suggestive of bone gain. Even though the current study used linear measurements to assess bone levels, our findings are comparable to the study by Hwang *et al.*, (2008) and Dubrez *et al.*, (1990) with 89.1% of sites displaying vertical bone loss in the test group having bone gain and 82% bone gain recorded in the control group.

The results of the current study illustrate more radiographic bone gain was observed in the SRD-with perioscope group as indicated by the higher mean RBL as compared to SRD-only. At twelve months, the test group had an improved RBL with a higher frequency of RBL between 0.5 mm and 1.0 mm and 1.0 mm to 1.5 mm as compared to the control group, inferring that the test group had more sites with radiographic bone gain in this range as compared to the control group.

Figure 5.7 and 5.8 shows displays single and multi-rooted teeth with areas of radiographic bone gain following SRD-with perioscope.

The systematic review by Nibali *et al.*, in 2015 supported non-surgical techniques for managing periodontal disease. This review found that radiographic improvements in intrabony defects following non-surgical therapy are the same range as observed in studies on periodontal regeneration (median 3.3 mm in radiographic intrabony defect gains) (Nibali *et al.*, 2015a). In 24 patients with advanced periodontitis, mean radiographic changes from the CEJ to the alveolar crest was assessed at six and 24 months following guided tissue regeneration. This study reported a 0.37 ± 1.52 mm mean change in RBL at six months and 0.29 ± 1.54 mm change at 24 months (Klein *et al.*, 2001). These changes in mean RBL is lower than reported in the current study suggesting that minimally invasive methods of managing periodontal disease may be equally as effective.

Analysis of mean change in RBL for single and multi-rooted teeth were included in this study. Changes in mean RBL for single and multi-rooted teeth for both groups were observed at twelve months. No statistical differences were found between groups for single rooted teeth; however, for multi-rooted teeth, a significant difference was reported with the test group showing more radiographic bone gain for multi-rooted teeth (0.83 ± 0.45 mm) as compared to the control group (0.46 ± 0.36 mm) ($p < 0.05$). In addition, for multi-rooted teeth the test group had a higher frequency of RBL from 0.5 mm to 1.0 mm and 1.5 mm to 2 mm as compared to the control group. The control group had no changes in RBL from 1.5 mm to 2.0 mm. Increases in alveolar bone density for single and multi-rooted teeth was described in the study by Hwang *et al.*, (2008). The results of this study showed that multi-rooted teeth displayed greater increases in alveolar bone density. However, statistical significance was not reached ($p > 0.05$) (Hwang *et al.*, 2008). There are no studies that have assessed the frequency and range of RBL.

The current study supports that SRD can result in positive changes occurring in the alveolar bone following therapy, similar to findings in other studies (Dubrez *et al.*, 1990; Hwang *et al.*, 2008; Nibali *et al.*, 2015b; Nibali *et al.*, 2011; Okano *et al.*, 1990). Continued SPT can also result in stable alveolar bone height as demonstrated in a recent study by Killeen *et al.*, (2018) where patients received SPT for 24 months

(Killeen *et al.*, 2018). Findings of the retrospective study on teeth with furcation involvement, supported patient compliance with ongoing maintenance, with compliant patients losing less bone in furcation sites as compared to non-compliant patients (Kim *et al.*, 2017). For the current study, all patients were seen every three months for SPT during the research period which could have contributed to the stability of alveolar bone levels.

Like any diagnostic tool, the taking and analysing of periapical radiographs has its limitations. As discussed previously, there were many issues faced with exact replication of radiographs taken at twelve months which in turn affected the number of radiographs analysed. These included patient's intolerance to positioning stents, damage to putty and movement of radiographic sensors during exposure. Some of these limitations were also described in the study by Schmidt *et al.*, (1996). It is also important to remember that tooth wear and tooth movement can occur, and customised stents may not provide an accurate replication of radiographs previously taken (Zappa *et al.*, 1991). Superimposition of images can also mask osseous changes (Hamanaka *et al.*, 2013).

To reduce inter-examiner variability, all radiographic measurements were done by one person (MN) and repeated for each site. Statistical results revealed, only minimal differences between the measurements with an excellent agreement between measurements. However, measurement errors can occur with overestimation and underestimation of distances. Estimation of the exact position of the CEJ can also be affected by restorations. In addition, one must bear in mind that peri-apical radiographs provide two-dimensional image of a three-dimensional objects or areas which can lead to problems with validity, accuracy and precision (Hausmann, 2000). Studies have also found that computer-assisted linear distances on radiographs actually underestimate the amount of interproximal bone loss when compared to surgical measurements (Eickholz *et al.*, 2000). Similar findings were found in other studies where linear measurements underestimated the changes in the alveolar bone (Toback *et al.*, 1999; Tonetti *et al.*, 1993).

New software programmes utilizing digital subtraction radiography allows us to analyse changes in periodontal hard tissues following periodontal therapy. This system has reduced the need for standardisation, provided that the radiographs are well-

angulated. It also allows the analyses of serial radiographs (Hwang *et al.*, 2008). Digital subtraction radiography has shown to be reliable with a detection of 5% change in the mineral mass per unit volume (Ortman *et al.*, 1985). However, these changes in density are also subjective.

Computer-Assisted Densitometric Image Analysis System (CADIA) measures the light transmitted through the radiograph and converts it to a gray scale image. This is then transmitted to a computer programme where measurements can be computed. CADIA is similar to subtraction radiography; however, it was found to be more reproducible and accurate and is able to follow periodontal disease progression (Bragger *et al.*, 1988).

A recent study by Lin *et al.*, (2017) focussed on identifying the CEJ and used an automated length-based alveolar bone loss measurement system and suggested that this method can be used successfully to estimate degree of horizontal bone loss (Lin *et al.*, 2017).

CBCT technology is also valuable for assessment of furcation areas and intrabony defects and can assist with treatment planning and prognosis of teeth. However, the radiation dose needs to be considered and routine use of CBCT technology may not be advised (du Bois *et al.*, 2012). CBCT was found to be more precise and accurate when compared to intra-oral radiography in the study by Grimard *et al.* (Grimard *et al.*, 2009). The newer machines allow a field of view to be taken, which limits the radiation dose to patients. These changes in technology may allow more use of CBCT imaging for management of periodontal disease. However, cost of software programmes utilized with these programmes have to be considered (Hamanaka *et al.*, 2013).

Even though there are methods and techniques available to get a more accurate description of alveolar bone changes in subjects with periodontal disease, the methods used for the purposes of this study were able to provide a clear depiction of changes in RBLs for the study population. Studies have validated the use of linear measurements. The method used which included magnifying the radiographic image and measuring the distance from the alveolar bone crest to the CEJ showed better reproducibility of measurements compared to measuring alveolar bone level in relation to root length or measuring the distance of the alveolar crest and the crown tip as a

proportion of the total length of the tooth as described by Albandar and Abbas (1986) (Albandar *et al.*, 1986). Using a simple modification to the Rinn holder can provide a significant degree of standardization and allows comparison of serial radiographs (Carpio *et al.*, 1994). The method of standardization of intra-oral radiographs using an occlusal splint and film-holder in the study by Hamanaka *et al.*, (2013) also supported the use of this method as consistent reproduction of geometric exposure parameters were reported, making it possible to gather information from these radiographic images (Hamanaka *et al.*, 2013).

Analysing radiographic changes for longer than 12 months and being able to track osseous changes is an important component of long-term management of patients with periodontal disease. Long term studies have shown that bone loss can occur following therapy and supportive periodontal maintenance. The retrospective study by Greenstein *et al.*, in 2009 compared radiographs taken at initial appointments and during periodontal maintenance (6 to 16 years; mean: 8.83 years) and reported that additional angular and horizontal bone loss of 5.56% and 3.88% was found in these patients (Greenstein *et al.*, 2009). Similarly, 95 patients who had periodontal therapy and maintenance for a mean of 7 years, had bone loss after surgery; however more than half of the patients had no change on bone height between examinations (Becker *et al.*, 1984). No change in mean alveolar bone height were reported in the study by Lindhe and Nyman (1984) study who assessed 61 patients following periodontal therapy and maintenance for 14 years (Lindhe *et al.*, 1984). We are aware that bone loss can occur despite periodontal maintenance, therefore long-term monitoring of osseous changes is vital.

Minimally invasive techniques have been advocated to minimize patient discomfort and maximize healing potential (Nibali *et al.*, 2015b). One can presume that the use of the perioscope as an adjunct to SRD has resulted in more radiographic bone gain as compared to SRD alone. SRD-with perioscope was also more successful in gains in RBL in multi-rooted teeth which are normally more difficult to manage non-surgically. One can speculate that more bone fill occurred when using the endoscope for non-surgical management of periodontal disease as the direct visualization technique aids in more effective removal of subgingival calculus deposits which in turn has a positive impact on the healing of the periodontal tissues. However, true confirmation of alveolar

bone changes is through histology or open flap measurements which is not a practical approach for routine assessment.

Despite the limitations of the current study, this study brings evidence that less invasive non-surgical periodontal therapy with the use of the perioscope has been found to be advantageous in improving mean RBLs resulting in radiographic bone gain and should be considered for use with patients with periodontal disease. However, more longitudinal studies are required to validate the results of this study and to compare the use of the perioscope with conventional non-surgical debridement on osseous changes. There are many advances in radiography methodology and techniques for analysing of radiographs that need to be researched and included in future studies. Changes in radiographic density and mineral bone change should also be considered for future research.

**Figure 5. 7 Standardised Radiograph of single rooted teeth before and after SRD
-with perioscope**



**Figure 5. 8 Standardised Radiograph of multi- rooted teeth before and after
SRD -with perioscope**



CHAPTER 6

POST-TREATMENT FOLLOW UP

This chapter provides information on post-treatment follow-up for all study participants.

Patients were presented with a long-term treatment plan prior to commencement of non-surgical treatment. At the twelve-month re-evaluation appointment, all participants were given recommendations regarding treatment options to further stabilise the periodontium as well as for any other specialised dental treatment that they may require.

Practice policies were followed with regards to post-treatment follow-up for patients who completed the study. The following were taken into consideration for further management; the patient's medical history, their oral hygiene status assessed by using a PI, anatomical considerations, cost to the patient and the patient's willingness to want to proceed with further treatment. With regards to periodontal surgery, patient's oral hygiene and home care procedures were assessed with a PI of below 30% required. Not all sites with periodontal pockets >5 mm were considered for periodontal surgery. In some patients with pocket depths 5-6 mm, patients will be continually monitored to determine if repeated non-surgical periodontal therapy and supportive periodontal maintenance is adequate. Perioscopy was recommended to those patients with residual pocketing mainly around the incisor and canine teeth in the control group.

In a few cases, periodontal surgery was indicated as removal of the excess gingival tissue will improve access for home care procedures. In some, but not all cases where osseous defects were evident, and where the gingival architecture resulted in difficulty for the patient to maintain adequate plaque control, and in addition was difficult to access during SPT appointments, osseous surgery was indicated. Alveolar bone was re-sculptured either by ostectomy or osteoplasty depending on what was observed after reflecting the mucogingival flaps. In a few cases, if teeth were deemed with questionable to poor prognosis, these sites were not considered for surgery and will be maintained during SPT appointments. If non-surgical management was not successful in these areas and bleeding and inflammation still persisted and patients still wanted to keep these teeth, perioscopy would be considered to enable direct visualization and removal of any residual deposits of calculus. Participants with residual pocketing and bleeding upon probing and were awaiting orthodontic advice,

perioscopy was also suggested for these patients. Here again, cost to the patient was considered as the cost of perioscopy is lower than periodontal surgery.

6.1 Post-treatment Follow -Up

Table 6.1 describes the type of treatment and recommendations made to participants after completion of the research study.

Following completion of the study, which included SRD-alone and SRD-with perioscope, as well as three monthly supportive periodontal maintenance for twelve months, the following recommendations were made:

- All thirty-eight participants were advised to continue with supportive periodontal maintenance, and all are currently in the maintenance programme.
- Periodontal surgery was recommended as a treatment option for nine of the nineteen (47%) participants in the control group, and in the test group, five participants (26%) were suggested for periodontal surgery. Pocket reduction and osseous surgery were recommended for six of the ten participants in the control group. Osseous surgery including resection or distal wedge surgery were suggested for two of the nine participants in the control group. One participant in the control group required gingival recontouring and osseous surgery. In the test group, osseous surgery was recommended for four of the five participants to manage the intra-bony defects and furcation areas. For one of the participants in the test group, periodontal resection surgery was recommended to remove excess tissue to create better access for home care.
- Perioscopy was suggested for three participants in the control group. Two of the three participants had residual pocketing in the anterior teeth. Perioscope was considered instead of surgery due the aesthetics and possibility of gingival recession that may occur with surgical intervention. For one participant, who was waiting to proceed with orthodontic treatment, perioscope was recommended as a treatment option to manage residual pocketing until a decision was made regarding further treatment.
- Two participants in the control group were referred for orthodontic assessment. This recommendation was part of the long-term treatment plan for the patients.
- Implant placement was part of the long-term treatment plan for six participants (five in the test group and one in the control group). Once stability of the

periodontium was achieved, patients were advised to seek assessment for placement of dental implants. Three of the five participants in the test group are in the process of implant placement. One participant in the test group has completed implant placement. The other two are still in the planning phase. One participant in the control group and one in the test group have decided not to proceed with implant placement at this stage.

Table 6. 1 Post-treatment follow up

Post Treatment Follow - Up	Control	Test
Supportive Periodontal Maintenance	19	19
Perioscopy	3	0
Periodontal Surgery	9	5
Orthodontic Assessment	2	0
Implant Assessment	1	5

6.2 Periodontal Health

The proceedings of the joint workshop held by the AAP and EFP considered the inclusion of “periodontal health” which is “the state free from inflammatory periodontal disease” (Lang *et al.*, 2018). Periodontal health can be restored to an anatomically reduced periodontium by reducing inflammation and improving clinical health. Periodontal disease stability is defined “as a state in which periodontitis has been successfully treated through control of local and systemic factors, resulting in minimal BOP, optimal improvements in PPD and attachment levels, and a lack of progressive destruction”. Periodontal disease remission/control is defined “ as a period in the course of disease during which treatment has resulted in reduction but not total resolution of inflammation with some improvements in PPD and attachment levels” (Lang *et al.*, 2018).

Assessment of the current periodontitis status was made as follows:

- Patients that were categorised as “Currently stable” with BOP <10% and PPD $\leq$ 4 mm with no BOP at 4 mm sites
- Patients that were “Currently in remission” had BOP $\geq$ 10%, PPD $\leq$ 4 mm, no BOP at 4 mm sites
- Patients that were considered “Currently unstable” had PPD $\geq$ 5 mm or PPD $\geq$ 4 mm & BOP.

When considering BOP percentages for the study population, 10 of the 19 patients in the control group had a BOP <10% at twelve months, while 17 of the 19 patients in the test group had BOP<10%.

However, the new assessment of periodontal stability, takes into consideration the PPDs as well as BOP. When examining the percentages of sites with PPDs 1-4 mm, four patients in the test group had 100% of sites between PPDs 1-4 mm with all four patients having less than 10% BOP. These four patients in the test group can be categorized as “currently stable”. In the control group, 1 patient was classified as “currently stable” with 100% of sites with PPDs 1-4 mm and less than 10% BOP. (Table 6.2)

There were no patients in either of the treatment groups that were grouped as “currently in remission” as there were a few persistent pockets greater than 4 mm in both groups even though BOP was less than 10% in some of these patients. The SRD-with perioscope had less than 5% of sites with PPDs >4 mm at twelve months (mean percentage of PPDs 1-4 mm (95.62 $\pm$ 4.6%)) as compared to the control group with about 10% of sites with PPDs >4 mm (mean percentage of PPDs 1-4 mm (89.87 $\pm$ 7.9%)).

Table 6. 2 Periodontal Status at twelve months for control and test groups

Group	Control (n=19)	Test (n=19)
Number with BOP<10%	10	17
Number with BOP>10%	9	2
Number with PPD 1-4 mm	1	4
Currently Stable	1	4
Currently in Remission	0	0
Currently Unstable	18	15

CHAPTER 7

FINAL COMMENTS AND CONCLUSION

The overall aim of this study was to compare endoscope SRD with traditional SRD in reducing clinical and inflammatory parameters in a 12-month period.

It was hypothesized that endoscope-aided SRD would result in greater reduction in mean PPDs as compared to SRD-only at the three-month re-evaluation appointment. It was also hypothesized that PPDs in the test group will continue to decrease even further or be sustained at the 12-month re-evaluation appointment.

The results of this investigation show that both SRD and SRD with aid of an endoscope were both effective in reducing mean PPDs following therapy. At three months, even though no significant differences between mean PPDs were observed between the groups, the mean reduction in the PPDs was higher in the SRD-only group as compared to the SRD-with perioscope group. This could be related to the higher mean PPDs in the control group at the start of the trial. At twelve months, the SRD-with perioscope group had more reductions in mean PPDs as compared to the SRD-only group and these differences were statistically significant. This suggests that healing and maturation in the test group was better at twelve months. Studies have suggested that conventional SRD is effective in reducing PPDs <6 mm, with deeper pockets having more pocket depth reduction and gains in clinical attachment levels with surgical intervention (Heitz-Mayfield *et al.*, 2002). For the current study, it was found that the adjunctive use of the endoscope was more effective than conventional SRD in reducing PPDs 7-9 mm at three and twelve months with differences being statistically significant. This direct visualisation technique may provide an alternative option to manage deeper pockets. In addition, the adjunctive use of the endoscope resulted in a significantly higher percentage of PPDs 1-4 mm ($95.62 \pm 4.6\%$) as compared to the control group ($89.87 \pm 7.9\%$).

It was hypothesized that endoscope-aided SRD would result in less probing attachment loss, greater percentage reduction in bleeding and plaque scores. Improvements in PAL were observed with both treatment methods at three and twelve months with no statistically significant differences observed between the test and control groups. More attachment gain was seen with PAL 7-9 mm and 10+ mm in the test group with results bordering on significance. More controlled trials with larger sample sizes are required to test the efficacy of the periodontal endoscope in achieving more gains in attachment levels.

When examining the change in gingival recession from baseline to twelve months in both groups, gingival recession was also found be lower in the test group as compared to the control group. The use of the direct visualization technique may result in less tissue trauma and less gingival recession. It can be a suggested method of debridement especially in aesthetic zones and in patients with thin biotype where gingival recession should be kept to its minimal.

Both BOP and PI decreased in both groups post-therapy and at twelve months. A statistically significant lower mean PI% was reported in the test group as compared to the control group at nine and twelve months. The mean BOP in the test group was significantly lower than the control group at twelve months. The lower mean BOP in the test group could be attributed to the direct visualization technique. The technique of being able to view the magnified subgingival deposits and remove them at the same time could have resulted in more successful removal of these deposits and hence less residual inflammation and BOP. Studies conducted utilising the endoscope have supported this concept of less inflammation following debridement with the endoscope. The reduction of BOP following debridement with the endoscope has been reported in many studies (Blue *et al.*, 2013; Checchi *et al.*, 2009; Hou.Y, 2016; Liao *et al.*, 2016; Stambaugh *et al.*, 2000). Wilson *et al.* showed no histologic signs of inflammation following a single treatment of SRD with the endoscope. A statistically significant relationship between subgingival calculus covered with biofilm and inflammation of the pocket wall measured by the redness of the pocket epithelium were also reported (Wilson *et al.*, 2008a; Wilson *et al.*, 2008b) . For the current study, the endoscope was not used to check sites with BOP for presence of residual calculus. This should be considered for future studies.

“Periodontal stability” as per the 2017 periodontal classification takes into consideration the PPDs as well as BOP. When examining the percentages of sites with PPDs1-4 mm, four patients in the test group had 100% of sites between PPDs 1-4 mm and less than 10% BOP. These four patients in the test group can be categorized as “currently stable”. In the control group, one patient was classified as “currently stable” with 100% of sites with PPDs 1-4 mm and less than 10% BOP.

It was hypothesized that a greater reduction in bacterial counts would be seen at three and twelve months in test group as compared to the control group. However, the results of the current study found no significant differences in levels and numbers the eleven pathogens examined and the bacterial complexes between the test and control group post-SRD and twelve months. Both treatment modalities resulted in decreases in numbers of periodontal pathogens including *P. gingivalis*, *T. forsythia*, *T. denticola*, *P. intermedia*, *P. micros*, *E. nodatum* and *E. corrodens* post-therapy and at twelve months with numbers remaining below pre-treatment levels. Research has shown that bacterial recolonization can occur immediately after treatment (Cugini *et al.*, 2000; Haffajee *et al.*, 1997b; Harper *et al.*, 1987; Shiloah *et al.*, 1994) and this was observed with *A. actinomycetemcomitans*, *C. rectus* and *Capnocytophaga sp.* (*C. sputigena*, *C. gingivalis*, *C. ochracea*) in this study. These pathogens decreased post-therapy in both groups but increased at twelve months. *F. nucleatum* increased in both groups post-SRD and then was found at lower levels at twelve months. When examining the complexes of bacteria, the red and orange complex had lower numbers in the test group at twelve months, with the control group having lower numbers of the green and orange-associated complexes; however, no significant differences were noted between the groups.

There were many limitations with the microbial test used for this study, making it difficult to make clear inferences about changes in pathogen levels and numbers post-therapy and at twelve months. The Micro-IDent test by Hain Lifescience is a simple chairside test, however results are semi-quantitative. The samples taken were pooled and included persistent deep pockets. This could have potentially influenced the levels and percentages of the pathogens as reflected in the results. For future studies, analysis of individual sites would be recommended as this will allow correlation analysis with clinical parameters. Bacteria need to reach specific thresholds before they can cause disease. Sampling of individual sites and determination of these thresholds may have been more beneficial in assisting with predicting disease progression. Future studies should consider also include the examination of the synergistic relationship and co-aggregation of bacteria.

It was hypothesized that SRD-with perioscope would result in statistically significant radiographic bone gain as compared to SRD only. For the current study, radiographic

analyses revealed a statistically significant change in mean RBL with the test group having a more radiographic bone gain as compared to the control group. Teeth were also analysed as single-rooted and multi-rooted teeth. No differences were observed with regards to single-rooted-teeth. However, for multi-rooted teeth the test group had more radiographic bone gain as compared to the control group. In addition, the test group had a higher frequency of RBL between 0.5 mm and 1.0 mm and 1.0 mm to 1.5 mm as compared to the control group, inferring that the test group had more sites with radiographic bone gain in this range as compared to the control group. These results are suggestive that the SRD-with perioscope was more successful in improving RBL and resulting in more sites with bone gain at twelve months as compared to SRD-only.

However, difficulties were experienced with standardising of radiographs and positioning stents, which resulted in exclusions of some of the radiographs. The analysis of RBL included linear measurements which could potentially result in underestimation or overestimation of measurements. In addition, the exact position of the CEJ can be difficult to determine due to restorations. Superimposition of anatomical structures can also mask osseous changes. Measurement of the angle of the vertical defects were not included in this trial and should be considered for future studies. Advances in radiographic technology including digital subtraction radiography or use of CBCT may simplify tracking radiographic changes following therapy and should be considered for future research. Analysis of clinical parameters did not include analysing single and multi-rooted teeth. This may have been valuable to make comparisons with radiographic analyses.

There were some issues relating to bias for the current study. One clinician was responsible for all clinical data collection and treatment. However, blinding of the clinician was not possible in a private practice setting. Consistency of data collection to reduce measurement errors and consistency of treatment were also deemed important. More than one clinician would have been necessary if blinding was to occur and due to training and manpower issues, this was not possible.

With regards to post-treatment f/up, periodontal surgery was recommended as a treatment option for nine of the nineteen participants in the control group, and in the

test group, five participants were suggested for periodontal surgery. Perioscopy was suggested for three participants in the control group. This research study was carried out in a private specialist practice and compliance to continue with ongoing SPT was excellent with all thirty-eight participants enrolled in the SPT programme.

7.1 Conclusion

In summary, the results of this study have demonstrated positive outcomes with clinical, microbiological and radiographic changes following non-surgical periodontal therapy in a cohort of patients with chronic moderate to advanced periodontal disease. Significantly greater decreases in percentage of PPDs 7-9 mm was observed at three and twelve months in SRD-with perioscope group when compared to the SRD-only group. Lower mean PPDs, BOP% and PI% were seen in the test group at twelve months compared to the control group. Gingival recession was significantly lower in the test group at twelve months. More radiographic bone gain was observed with the use of the endoscope as compared to conventional SRD. This finding included multi-rooted teeth.

Perioscopy is a technique sensitive procedure with a high learning curve. However, the positive clinical and radiographic outcomes observed in this clinical trial with the use of the endoscope supports further investigation to understand the value of direct visualization techniques in a clinical setting. An option to treat periodontal disease in a less invasive way may be advantageous compared to a surgical approach and with more research and improvements in visualization techniques for non-surgical management of periodontal disease, this method could provide an alternative for surgery. Larger, prospective and controlled studies are recommended to test the efficacy of this technology. Training of more oral health professionals to use minimally invasive techniques such as periodontal endoscopy is recommended. This added skill will provide both operators and patients with an alternative approach to manage periodontal disease non-surgically.

It is hoped that future studies will test these hypotheses facilitated by developing technologies.

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