CLINICAL, MICROBIOLOGICAL AND IMMUNOLOGICAL RESPONSES TO TWO NON-SURGICAL PERIODONTAL TREATMENT MODALITIES

Danae Anastasia Apatzidou B.D.S. (Greece)

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University of Glasgow

Periodontology and Oral Immunology Research Group,
Glasgow Dental Hospital and School,
University of Glasgow

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

To my beloved nephew Andreas Eliopoulos

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Ὁ ΒΙΟΣ βραχὺς, ἡ δὲ τέχνη μακρὴ, ὁ δὲ καιρὸς ὀξὺς, ἡ δὲ πεῖρα σφαλερὴ, ἡ δὲ κρίσις χαλεπή. Δεῖ δὲ οὐ μόνον ἑωυτὸν παρέχειν τὰ δέοντα ποιέοντα, ἀλλὰ καὶ τὸν νοσέοντα, καὶ τοὺς παρεόντας, καὶ τὰ ἔξωθεν.

Vita brevis; ars longa; occasio celeris; experimentum periculosum; judicium difficile. Oportet autem non modo se ipsum exhibere quæ oportet facientem, sed etiam ægrum, et præsentes, et externa.

Life is short; art is long; opportunity fugitive; experience delusive; judgment difficult. It is the duty of the physician not only to do that which immediately belongs to him, but likewise to secure the co-operation of the sick, of those who are in attendance, and of all the external agents.

Declaration

This thesis is the original work of the author.

Danae Anastasia Apatzidou

Abbreviations

A. actinomycetemcomitans	*Actinobacillus actinomycetemcomitans*
A. a.	*Actinobacillus actinomycetemcomitans*
AL	attachment level
ANOVA	analysis of variance (one-way)
B. forsythus	*Bacteroides forsythus*
B. f.	*Bacteroides forsythus*
BAS	baseline
BOP	bleeding on probing
BSA	bovine serum albumin
CB	coating buffer
CEJ	cemento-enamel junction
CFU	colony forming units
C. gracilis	*Campylobacter gracilis*
CL	leukocyte chemiluminescence
cm	centimetre
C. rectus	*Campylobacter rectus*
DA	Danae Apatzidou
DNA	deoxyribonucleic acid
E. corrodens	*Eikenella corrodens*
ELISA	enzyme-linked immunosorbant assay
E. nodatum	*Eubacterium nodatum*
EU	ELISA units
Fdis	one-stage full-mouth disinfection
FM-SRP	full-mouth scaling and root planing
F. nucleatum	*Fusobacterium nucleatum*
FRp	full-mouth root planing
GCF	gingival crevicular fluid
GI	gingival index
GLM	general linear model
HIV	human immunodeficiency virus
IB	incubation buffer
ID_{50}	50% inhibitory dose

IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
M	molarity
MGI	modified gingival index
mm	millimetre
ng/ml	nanograms per millilitre
OD	optical density
OHI	oral hygiene instructions
PBS	phosphate-buffered saline
PBST	phosphate-buffered saline with Tween 20
PCR	polymerase chain reaction
PD	pocket depth
P. gingivalis	*Porphyromonas gingivalis*
P. g.	*Porphyromonas gingivalis*
P. intermedia	*Prevotella intermedia*
P. i.	*Prevotella intermedia*
PI	plaque index
P. micros	*Peptostreptococcus micros*
P. nigrescens	*Prevotella nigrescens*
Q-SRP	quadrant scaling and root planing
R1	reassessment 1
R2	reassessment 2
RAL	relative attachment level
rRNA	ribosomal ribonucleic acid
sd	standard deviation
sec	seconds
SEM	standard error mean
S. intermedius	*Streptococcus intermedius*
S. mutans	*Streptococcus mutans*
SRP	scaling and root planing
Sup	suppuration
T. denticola	*Treponema denticola*
T. d.	*Treponema denticola*

TMB	tetramethylbenzidine
TNF	tumor necrosis factor
V. parvula	*Veillonella parvula*

Summary

The current study compared the clinical, microbiological and immunological parameters of chronic periodontitis patients that received two different treatment strategies: one-day full-mouth scaling and root planing (FM-SRP) versus quadrant scaling and root planing at two-weekly intervals (Q-SRP). In addition, the effects of smoking on the periodontal status, subgingival microflora and humoral immune response were examined.

Clinical measurements, subgingival plaque samples, gingival crevicular fluid (GCF) and sera were collected from 42 patients with moderate to advanced chronic periodontitis before and after treatment over a period of six months. Patients were randomly allocated into two treatment groups and received FM-SRP or Q-SRP. Polymerase chain reaction (PCR) was used to determine the presence of *P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia*, *T. denticola* and *B. forsythus* in plaque. Enzyme-linked immunosorbent assay (ELISA) was used to examine systemic and local antibody titres to these bacteria and thiocyanate disassociation was used to determine antibody avidity.

Q-SRP and FM-SRP were found to be equally efficacious periodontal treatments. Therefore, the clinician should decide which treatment strategy to choose on a practical basis. No evidence emerged from this study to document a strong immunological reaction elicited by FM-SRP, as was speculated by Quirynen et al. (1995). In fact, during therapy the host responses of patients in both treatment groups followed a similar pattern. In general, both treatments resulted in significant clinical improvement and reduced titres of antibodies, but which were of similar avidity for the majority of the tested organisms. These changes paralleled marked reductions in the site and subject prevalence of putative periodontopathogens. In addition the present data do not support the hypothesis that, during the active phase of quadrant root planing therapy, the treated sites are re-infected with organisms that are possibly transmitted from the remaining untreated pockets and from other intra-oral niches (Quirynen et al. 1995). In contrast, the commencement of quadrant root planing and meticulous oral hygiene measures had a significant positive effect on the periodontal conditions of the remaining untreated quadrants. Nevertheless, this clinical improvement was smaller compared to changes seen after the completion of mechanical debridement. Despite the fact that FM-SRP

resulted in significantly higher pain scores and more patients taking analgesics compared to Q-SRP, it still seemed to be well tolerated by patients.

Smoking had a significant adverse effect on the inflammatory response to the bacterial challenge and on the treatment outcome. The outcome of each treatment strategy (Q-SRP and FM-SRP) was affected significantly by smoking over the course of treatment, having a significant impact on pocket depth (PD) and relative attachment level (RAL) of selected sites. A smaller PD reduction and less gain in RAL were noted after both treatment strategies at selected sites of smokers compared with those of non-smokers. Smoking appeared to modify both local and systemic host responses by producing lower levels of GCF and serum antibodies, respectively, but appeared to have no significant effect on the subgingival microflora.

This study associated systemic antibody levels with the presence of homologous organisms in subgingival plaque. Furthermore, the present study showed that subjects with less severe disease and a better clinical outcome retained higher levels of serum antibodies compared to patients who had more advanced disease and a moderate clinical outcome. These findings confirm the suggestions from other studies that antibodies are protective against the progression of periodontal disease. GCF antibody titres were low compared to serum antibody levels, which agrees with previous findings and a large site-to-site variability was evident, making interpretation of the results difficult. Nevertheless, it was apparent that deep pockets and sites that harboured a bacterial species gave rise to higher levels of GCF antibodies for that species.

CHAPTER 1

GENERAL INTRODUCTION

1. General Introduction

1.1 The periodontium in health and disease

Periodontium is a word of Greek origin, referring to the tissues that surround and support the tooth. It is comprised of 1) gingiva, 2) periodontal ligament, 3) root cementum and 4) alveolar bone.

In health, the gingiva is pink in colour, often stippled in appearance, firm in texture with no bleeding on probing. The healthy gingival margin has a scalloped outline. The oral epithelium which covers the gingiva and faces the oral cavity is keratinised and is continuous with the sulcular epithelium and the junctional epithelium, which line the inner surface of the gingival sulcus. The junctional epithelium provides the contact with the tooth surface *via* hemidesmosomes and, unlike the oral and sulcular epithelium, is not keratinized and lacks epithelial ridges projecting into the connective tissue, called rete pegs. The connective tissue, which is a major component of the gingiva and of the periodontal ligament, comprises collagen fibres, cells, vessels, nerves and extracellular matrix. Fibroblasts, mast cells and infiltrating leukocytes such as, macrophages, polymorphonuclear leukocytes, lymphocytes and plasma cells reside in the connective tissues even in health, but more so during periods of inflammation.

The periodontal ligament consists of cellular connective tissue and is highly vascular. It is defined topographically by the space between the root surface of the tooth and the lamina dura. Bundles of collagen fibres run through the ligament and attach the alveolar bone to the tooth. The dimensions (width and height) and characteristics of the ligament (including level of inflammation) determine the tooth mobility. The cells of the periodontal ligament are fibroblasts, osteoblasts, cementoblasts, osteoclasts, epithelial cells and nerves.

The root cementum shares many common features with the alveolar bone. It is a mineralized tissue that covers the root surface and, rarely, small parts of the tooth crown. The cementum has no vessels and nerves, is produced by the cementoblasts and, unlike the alveolar bone, it is characterised by continuous deposition and no remodeling. The fibres of the ligament that are embedded in the cementum and alveolar bone are called Sharpey's fibres.

The alveolar process supports the sockets of the teeth and consists of the compact bone, which lines the sockets, and the cancellous bone, which occupies the space between the sockets. The compact bone is perforated providing the periodontal ligament with blood and lymph vessels and nerves. The part of bone where Sharpey's fibres are embedded is called "bundle bone". The bone formation sites on the outer surface of the bone are lined with the osteoid and on top of this with the periosteum while, inside the bone, the bone marrows are lined with endosteum. Bone resorption and deposition is a continual process and is achieved by the osteoclasts and osteoblasts.

Gingival and periodontal inflammation has been classified into four categories based on clinical and histopathological findings: the initial and early gingival lesion, the established gingival lesion and the advanced lesion (Page and Schroeder, 1976). The initial and early lesions represent histopathologically the early stages of gingivitis, while the established lesion represents chronic gingivitis with a predominance of plasma cells. The advanced lesion reflects, histopathologically, the shift from gingivitis to periodontitis.

However, this classification does not seem to be suitable for the human model of disease progression. The human established lesion requires more than six months to develop severe plasma cell infiltration and plasma cells are still not the predominant cells at this stage of the disease (Brecx et al. 1988). Therefore, the above classification should be used for describing non-human periodontal disease patterns as suggested by other investigators (Kinane and Lindhe, 1997). Table 1 shows the new classification of periodontal disease based on histopathological evidence. Even in the early stages of gingival inflammation, that is not clinically detectable, the connective tissue and junctional epithelium are infiltrated by inflammation cells with an increase in the vascular components. "Clinically healthy gingiva", differs from "pristine gingiva" in that the latter shows little or no evidence of histologic inflammation. "Pristine gingiva" is almost impossible to see in everyday clinical practice and is considered to be an experimental condition.

1.2 Classification of periodontal diseases

In 1989, a classification system for periodontal diseases was developed by the American Academy of Periodontology at the World Workshop in Clinical Periodontics (Caton,

Table 1. Histopathologial evidence for the new classification of periodontal disease.

Clinical condition	**Histopathological condition**
Pristine gingiva	Histologic perfection
Normal gingiva	Initial lesion (Page and Schroeder 1976)
Early gingivitis	Early lesion (Page and Schroeder 1976). Few plasma cells.
Established gingivitis	Established lesion with no bone loss nor apical epithelial migration (plasma cell density between 10 and 30% of leukocyte infiltrate)
Periodontitis	Established lesion with bone loss and apical epithelial migration from the amelocemental junction (plasma cell density > 50%)

1989). Subsequently, a simpler classification was agreed at the 1st European Workshop in Periodontology in 1993 (Attström and van der Velden, 1994). More recently, the American Academy of Periodontology, in an International Workshop, revised the classification system for periodontal disease (Armitage, 1999) and this is illustrated in Figure 1 (Kinane, 2001). This classification system is used to describe different forms of periodontal diseases throughout this thesis.

1.2.1 Chronic periodontitis

The workshop participants in 1999 agreed that the term "adult periodontitis" should be replaced by "chronic periodontitis" in an attempt to discard the age-dependent nature of the adult periodontitis classification. This form of periodontitis is commonly found in adults, but also in adolescents (Papapanou, 1996). It has been characterised as a slowly progressive disease (Papapanou et al. 1989; Brown and Löe, 1993), although short periods of rapid progression were also noted in some patients (Socransky et al. 1984; Jeffcoat and Reddy, 1991).

1.2.2 Aggressive periodontitis

As has been stated, the new classification system (Armitage, 1999) revised previous terminologies that were age-dependent or were based on the rates of disease progression. Therefore, the previous designations of "prepubertal", "juvenile", and "rapidly progressive periodontitis" (Caton, 1989) or "early-onset periodontitis" (Attström and van der Velden, 1994) were replaced by the term "aggressive periodontitis". With the new classification "generalised aggressive periodontitis" replaces the older terms of "generalised juvenile periodontitis" and "generalised early-onset periodontitis" and "localised aggressive periodontitis" replaces the terms "localised juvenile periodontitis" and "localised early-onset periodontitis". The "prepubertal" forms of disease are described now as "generalised aggressive" or "localised aggressive periodontitis" that occur prepubertally.

1.2.3 Periodontitis as a manifestation of systemic disease

At the International Workshop (Armitage, 1999), the older term "periodontitis associated with systemic disease" (Caton, 1989) was retained. Among the systemic diseases that are closely associated with periodontitis, diabetes mellitus is not included, since there is not enough evidence to document a diabetes mellitus-associated form of

Figure 1

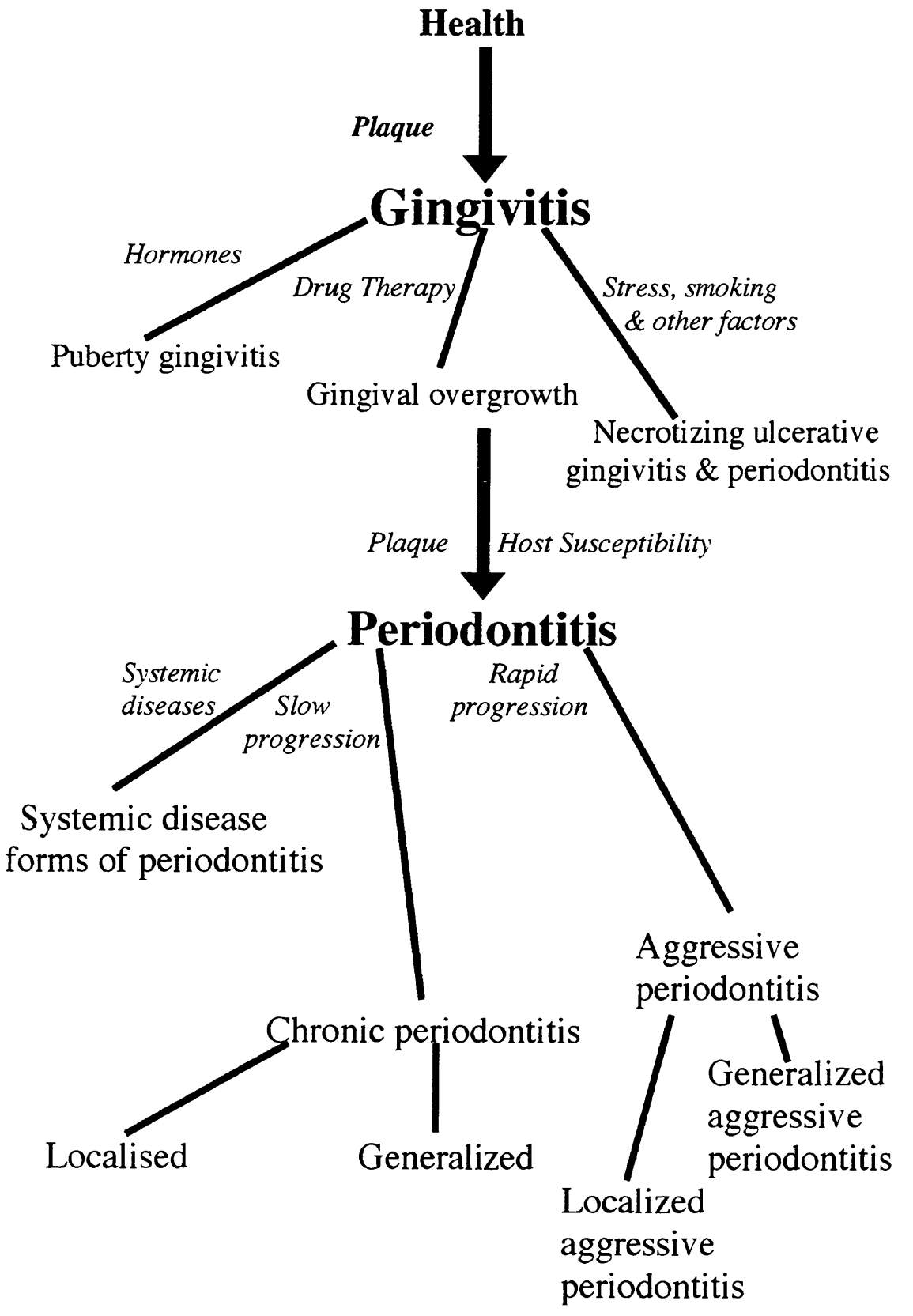


Figure taken from Kinane (2001). The latest classification for periodontal diseases, agreed at the 1999 International Workshop by the American Academy of Periodontology.

periodontitis. In addition, the new classification does not include a separate disease category for the impact of smoking on periodontitis.

1.2.4 Necrotising periodontal diseases

At present, there is not enough evidence to support the notion that necrotising ulcerative gingivitis and necrotising ulcerative periodontitis are different forms of disease. In addition, there is lack of data to clarify whether these clinical conditions are related to systemic diseases (e.g. HIV) or to other factors such as stress and smoking. Therefore, the new classification (Armitage, 1999), combined necrotising ulcerative gingivitis and necrotising ulcerative periodontitis in one disease category, designated as "necrotising periodontal diseases".

1.3 The pathogenic process

Periodontal pathogens cause disease after colonising subgingival sites and producing factors that induce direct or indirect destruction of the host tissues. In order to achieve this, the pathogen should be able to attach to the host tissues, multiply, compete with other subgingival species and finally escape from the host defence mechanisms. There are a number of difficulties a microorganism may encounter in colonising a periodontal pocket. The constant outflow of the gingival crevicular fluid (GCF) and the presence of saliva washing out the tissues tends to prevent the attachment of pathogens. In addition, the constituents of saliva and GCF have many innate and adaptive antimicrobial properties. The anatomy of the local tissues, desquamation of the epithelial cells and mechanical masticatory forces are innate processes that limit aggregation of dental plaque on the gingivae and provide defensive mechanisms against the pathogens.

As microorganisms attempt to escape the surveillance of the host immune responses, they are able to multiply and infect subgingival sites. The factors they produce irritate the local tissues and trigger an inflammatory process. Even during the early stages of periodontal disease, vasodilatation is evident and many lymphocytes and neutrophils (but few plasma cells) migrate through the capillaries and infiltrate the tissues (Seymour et al. 1983; Brecx et al. 1987). The cell infiltrates at this stage may occupy up to 15% of the connective tissue volume. As the inflammation advances, tissue breakdown (collagen fibres and matrix) and swelling become more severe and plasma cell populations increase. The dentogingival epithelium continues to proliferate and the rete

pegs extend deeper into the connective tissue. The junctional epithelium becomes detached from the crown surface, forming a periodontal pocket, and heavy leukocyte infiltration is present in the connective tissue and eventually in the gingival crevice. The junctional epithelium migrates apically and alveolar bone loss occurs, resulting in a denser and more anaerobic subgingival microbial flora. There is also a predominance of plasma cells, which account for over 50% of the connective tissue volume. Plasma cells are shown to out-number the other infiltrating cell types in periodontitits lesions (Garant and Mulvihill, 1972). While the destruction of the host tissues continues, increasing tooth mobility and drifting occurs and finally tooth loss may occur when the supporting structures are eventually destroyed.

1.4 Infectious aetiology of periodontal diseases

Periodontitis is a multifactorial inflammatory disease process, leading to destruction in the periodontium of the tissues supporting the teeth. It has been proven that oral bacteria and their products play an important role in the initiation and progression of periodontal diseases. This is documented by numerous investigations, which associate: 1) the successful use of antibiotics and antiseptic mouthrinses with the control of various forms of periodontitis, such as necrotising periodontal diseases (Mitchell and Baker, 1968; Winkler and Robertson, 1992), 2) optimal plaque control with the successful treatment of periodontal diseases (Lindhe and Nyman, 1975; Nyman et al. 1975), 3) microorganisms isolated from human periodontal pockets with periodontal destructive properties in animal experimental models (Jordan et al. 1972; Holt et al. 1988; Wray and Grahame, 1992), 4) immune responses elicited by the host with specific subgingival microorganisms (Ebersole et al. 1987; Ebersole and Taubman, 1994).

Despite the fact that the microbial aetiology of periodontal diseases has been accepted generally, there has been controversy. Questions arose as to why some teeth or even specific sites of certain teeth within the same individual underwent more severe periodontal destruction than others. Researchers sought to understand the characteristics of the microbial populations in dental plaques. The specific plaque hypothesis (Loesche, 1979) was introduced and, unlike the non-specific plaque hypothesis (Theilade, 1986), it suggested that specific periodontal pathogens are related to certain forms of periodontal diseases and when found in periodontal lesions they indicate increased risk of future periodontal breakdown. After refining sampling

strategies and laboratory techniques for the analyses of plaque samples the specific role of certain plaque organisms along with variations between subjects and sites within a subject have been documented. Each subgingival site designates a specific microenvironment, which may be at a different stage of disease severity from other affected sites within the same individual.

Many investigators have attempted to relate certain subgingival species to the aetiology of periodontal diseases. It appears that some periodontal lesions are caused by mixed infections and that the subgingival microbiota is in a state of continual flux with dynamic changes through the different stages of disease. This makes the definition of the role of a single species in the aetiology and pathogenesis of periodontitis difficult. Haffajee and Socransky, (1994) suggested the following subgingival species: *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, and *Treponema denticola* as putative periodontal pathogens. The authors summarised the literature relating to these putative periodontal pathogens with periodontal disease and attempted to rank specific organisms from strongest to weakest in their association with periodontal disease (Table 2).

Nevertheless, the presence of a putative pathogen subgingivally does not necessarily mean disease initiation and progression. Several studies have demonstrated that a combination of factors such as changes in the local environment, presence of virulent strains of periodontal pathogens in sufficient numbers, absence of beneficial species and host susceptibility contribute to disease progression (Socransky and Haffajee, 1992, 1993). In another study, it has been shown that although patients were considered clinically healthy, they still harboured *A. actinomycetemcomitans* and *P. gingivalis* post-therapy (Nieminen et al. 1995).

It has been shown that alterations in the local environment (temperature, osmolarity, iron concentration, pocket depth, inflammatory status) affect the expression of virulence factors and the ecology of the subgingival microbiota (Socransky and Haffajee, 1991a). Frequently, the suspected periodontal pathogens were present in healthy sites or in sites that responded well to treatment. This could be due to several reasons. For example, a particular species may not be pathogenic (despite its presence in diseased sites), the host may be resistant to a particular pathogen, the levels of this organism have not exceeded

Table 2. Ranking of suspected periodontal pathogens according to the strength of their relationship to periodontal disease.

Very strong	**Strong**	**Moderate**
A. actinomycetemcomitans	*B. forsythus*	*S. intermedius*
Spirochaete of necrotising ulcerative diseases	*P. intermedia*	*P. nigrescens*
P. gingivalis	*C. rectus*	*P. micros*
	E. nodatum	*F. nucleatum*
	Treponema species	*Eubactrerium* species
		E. corrodens

Table adapted from Haffajee and Socransky (1994).

A. actinomycetemcomitans: Actinobacillus actinomycetemcomitans

P. gingivalis: Porphyromonas gingivalis

B. forsythus: Bacteroides forsythus

P. intermedia: Prevotella intermedia

C. rectus : Campylobacter rectus

E. nodatum: Eubacterium nodatum

S. intermedius: Streptococcus intermedius

P. nigrescens: Prevotella nigrescens

P. micros: Peptostreptococcus micros

F. nucleatum: Fusobacterium nucleatum

E. corrodens: Eikenella corrodens

the threshold of the host susceptibility, or local environmental conditions may not favour pathogen replication and initiation of disease. Host susceptibility to periodontal pathogens is influenced by an impaired immune response related to hereditary and environmental factors such as living standards and smoking (Bergström and Eliasson, 1987a, 1987b; Williams et al. 1990; Safkan-Seppälä and Ainamo, 1992; Kinane et al. 2001). However, not all systemically diseased individuals or smokers have an altered subgingival microbiota or manifestations of periodontal disease. It appears that the simultaneous existence of a susceptible host, presence of pathogens, and an appropriate local environment are required in the initiation and progression of periodontal infection.

1.5 Association of the five putative periodontal pathogens with periodontal disease

1.5.1 *Actinobacillus actinomycetemcomitans*

Actinobacillus actinomycetemcomitans is a small, non-motile, gram-negative, saccharolytic, capnophilic, round-ended rod and its primary ecological niche is the periodontal pocket (Slots et al. 1980). This organism has been implicated in the causation of localised aggressive periodontitis (Mandell and Socransky, 1981; Zambon et al. 1983; Kornman and Robertson, 1985; Moore et al. 1985) and chronic periodontitis (Slots et al. 1980; Skaar et al. 1992; Mombelli et al. 1994a) and it is occasionally isolated from healthy subjects (Slots et al. 1980). Some individuals with no localised aggressive periodontitis (periodontally healthy subjects, chronic periodontitis patients and insulin-dependent diabetics) harbour *A. actinomycetemcomitans* (Zambon et al. 1983), which is in contrast to the findings of Mandel and Socransky (1981) who did not identify *A. actinomycetemcomitans* in chronic periodontitis and gingivitis patients. In addition, *A. actinomycetemcomitans* was not detected in four localised aggressive periodontitis patients (Loesche et al. 1985).

It has been suggested that *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* may be useful indicators of disease activity in adult patients with periodontitis (Bragd et al. 1987), although the absence rather than the presence of these bacteria has been shown to be a predictor of future attachment loss (Wennström et al. 1987c). Indeed, *A. actinomycetemcomitans* was detected significantly more frequently, and at higher levels, at sites which showed subsequent disease activity (Ebersole et al. 1995) and also, that these organisms are related to disease-active periodontitis lesions more closely than to

periodontal pocket depth (Slots et al. 1986). In another study, this organism was detected more frequently at sites without recession and it increased in sites with increasing pocket depth in both localised and widespread disease patients, but mean counts of this organism were higher in the localised forms of disease at all recorded pocket depths (Socransky et al. 1991b). Slots et al. (1980) pointed to a positive relation between *A. actinomycetemcomitans* and pocket depth, although data from another report showed that the mean proportions of this organism were found to be higher at intermediate pocket depths between 3 and 5 mm compared to depths above 5 mm (Wolff et al. 1985). The subjects in the latter study were not as severely diseased as those in the former study. However, there is evidence that there is no association between *A. actinomycetemcomitans* detection and clinical parameters in chronic periodontitis patients (Christersson et al. 1992; Doungudomdacha et al. 2001). The literature associating *A. actinomycetemcomitans* with periodontitis is inconsistent and a summary of the findings is that *A. actinomycetemcomitans* can be found both sub- and supragingivally. Additional research is required to confirm or deny its association with destructive periodontal lesions.

1.5.2 *Porphyromonas gingivalis*

Porphyromonas gingivalis is a gram-negative, anaerobic, non-motile, asaccharolytic rod that is usually seen in coccal or short rod morphologies. It is a member of the widely studied black-pigmented *Bacteroides* group of organisms. A low prevalence of *P. gingivalis* has been found in maintenance patients and in non-progressive chronic periodontitis patients (Loesche et al. 1985; Slots et al. 1986; Gmür et al. 1989). Higher frequencies and higher levels of this suspected pathogen were found in periodontal sites which were actively breaking down (Dzink et al. 1988). *P. gingivalis* predominates in subgingival plaques during the early stages of disease recurrence (Choi et al. 1990) and was found to have a relatively high prevalence in subgingival plaques from patients that responded poorly to treatment (refractory periodontitis patients) (Listgarten et al. 1993). It has been shown that *P. gingivalis* is elevated in periodontitis lesions and thrives in deep pockets (Gmür et al. 1989; Socransky et al. 1991b; Kojima et al. 1993; Kigure et al. 1995; Haffajee et al. 1998; Socransky et al. 1998; Ximenez-Fyvie et al. 2000b; Doungudomdacha et al. 2001) and is more prevalent at suppurating sites (Tanner et al. 1979; Socransky et al. 1991b; Hafström et al. 1994).

Gmür et al. (1989), showed that *P. gingivalis* was almost always detected in samples which harboured *Bacteroides forsythus*, but the latter organism was detected more frequently by itself and was found to colonise shallower pockets than *P. gingivalis*. This observation implies that either *B. forsythus* colonises earlier or in shallower lesions than *P. gingivalis*. Kojima et al. (1993) showed that as more *P. gingivalis* was identified in subgingival plaque samples, the percentage of deep sites or sites that bled on probing increased significantly. Nevertheless, this pathogen was also found in clinically healthy sites or it was absent in sites with deep pocket depths or sites with bleeding on probing. In addition, other studies showed the presence of this organism in dental plaque of children free of periodontitis (Barron et al. 1991; Ashimoto et al. 1996; Kamma et al. 2000). Ximénez-Fyvie et al. (2000b), demonstrated that the prevalence of *P. gingivalis* did not differ significantly between supra- and subgingival plaque deposits in subjects with chronic periodontitis, although counts and proportions of this organism were significantly higher subgingivally. Thus, there is a varied literature with respect to the relationship of *P. gingivalis* to periodontal diseases, but there appears to be a consistent trend that this species is frequently detected in advanced periodontitis lesions.

1.5.3 *Prevotella intermedia*

Prevotella intermedia is a gram-negative, short, round-ended anaerobic rod. *P. intermedia* is the second black-pigmented *Bacteroides* whose relationship to periodontal disease has been assessed by several studies. This organism has been associated mainly with necrotising periodontal diseases (Loesche et al. 1982), gingivitis (Slots et al. 1978; Ashimoto et al. 1996) and certain other forms of periodontal disease (Tanner et al. 1979; Dzink et al. 1983; Loesche et al. 1985). *P. intermedia* can be isolated from dental plaque as well as from the oral mucosa (Lie et al. 2001). *P. intermedia* was found at significantly elevated levels in active periodontal lesions (Dzink et al. 1985; 1988) and although it was closely related to progressive periodontitis, a high prevalence of it was also found in non-progressive sites (Slots et al. 1986). One reason for this could be that *P. intermedia* is associated with gingivitis as well as with periodontitis. This organism has been related to deeper periodontal lesions (Socransky et al. 1991b; Wolff et al. 1993; Ximenez-Fyvie et al. 2000b). Higher proportions of this organism were found in suppurating periodontal pockets (Dzink et al. 1983) and in abscess sites (Hafström et al. 1994). Nevertheless, there is conflicting evidence which does not

support an association between *P. intermedia* and clinical parameters in chronic periodontitis patients (Christersson et al. 1992; Doungudomdacha et al. 2001).

1.5.4 *Treponema denticola*

Treponema denticola is a gram-negative, anaerobic, helical-shaped, highly motile microorganism that is common in many periodontal pockets, and is classed as a spirochaete. Although *T. denticola* was found in dental plaque from children with no inflammatory periodontal disease (Barron et al. 1991; Ashimoto et al. 1996), this does not preclude its pathogenicity. A positive relationship between *T. denticola* and severe periodontitis has been demonstrated (Simonson et al. 1988, 1990). A further study confirmed that *T. denticola* was associated with severe chronic periodontitis and suggested that this organism may be useful as a prognostic marker for disease recurrence (Simonson et al. 1992). Adults with no periodontal disease were unlikely to harbour spirochaetes of any kind in dental plaque, whereas patients with periodontitis had spirochaetes in both supragingival and subgingival plaque (Riviere et al. 1992). In this study, spirochaetes including *T. denticola* were more common in diseased sites and in subgingival plaque than in healthy sites or in supragingival plaque.

Spirochaetes were predominant in plaques from patients with chronic periodontitis (Loesche et al. 1985) and *T. denticola* has been detected more frequently in deeper than shallower pockets (Simonson et al. 1988; Kigure et al. 1995; Haffajee et al. 1998; Socransky et al. 1998; Ximénez-Fyvie et al. 2000b). Nevertheless, this pathogen has also been detected in supragingival deposits, although in lower proportions than subgingivally (Ximénez-Fyvie et al. 2000b). The proportion of spirochaetes increases in the apical part of pockets in chronic periodontitis patients (Omar et al. 1990). However, a high proportion of spirochaetes were found at some coronal levels of the pockets and this could be due to the fact that either spirochaetes are inhabitants of this part of the pocket or they are translocated passively by the inflammatory exudate. This finding is in agreement with that of another study, which showed that most organisms isolated from pockets with recent bone loss including spirochaetes failed to show a difference between shallow and deep pocket depth (Tanner et al. 1984). A study by Kigure et al. (1995) found that *T. denticola* was predominant in the superficial layers of the subgingival plaque in advanced chronic periodontitis patients. At pocket depths of 2 to 4 mm and 4 to 6mm, *P. gingivalis* was located beneath the surface of subgingival

plaque while *T. denticola* was found in the surface of the samples. Nevertheless, the simultaneous presence of both bacteria was noticed at pocket depths exceeding 6 mm. In summary, the literature relating *T. denticola* to periodontal disease is consistent and supports a role for this fastidious anaerobe as a pathogen in periodontitis lesions.

1.5.5 *Bacteroides forsythus*

Bacteroides forsythus was first described in 1979, as a fusiform *Bacteroides* species (Tanner et al. 1979). This organism is slow-growing and is a gram-negative anaerobic, spindle-shaped, highly pleomorphic rod. *B. forsythus* was detected by immunofluorescence in subgingival microbial samples from patients with refractory periodontitis and was found to be the most prevalent species from an array of other micoorganisms (Listgarten et al. 1993). In addition, this organism was recovered more frequently from active than from inactive periodontitis lesions (Dzink et al. 1985; Tanner, 1986; Lai et al. 1987; Dzink et al. 1988). Studies have associated *B. forsythus* with untreated periodontal disease as well as recurrent periodontitis in treated patients and with relatively high numbers subgingivally (Lai et al. 1987). It has been demonstrated in several studies that this organism is more frequently detected in deeper than in shallower pockets (Gmür et al. 1989; Socransky et al. 1991b; Haffajee et al. 1998; Socransky et al. 1998; Ximénez-Fyvie et al. 2000b). However, *B. forsythus* was found in supragingival deposits although at lower counts and proportions than in subgingival plaque (Ximénez-Fyvie et al. 2000b). This species has also been detected in dental plaque from children with no periodontitis (Ashimoto et al. 1996; Kamma et al. 2000). Thus, although the evidence supporting *B. forsythus* as a periodontal pathogen is strong, more evidence is required to clarify the role of this species in the pathogenesis of periodontal diseases.

1.5.6 Coexistence of *Porphyromonas gingivalis, Treponema denticola* and *Bacteroides forsythus*

Socransky et al. (1998) demonstrated associations among bacterial species at periodontal sites using cluster analysis and community ordination techniques. The presence and levels of 40 species in subgingival plaque samples were determined using the checkerboard DNA-DNA hybridization technique. Five microbial complexes were identified and the species that comprise two of these complexes, the "red" and the "orange" are depicted in Table 3. The cluster of bacteria which consisted of *P.*

gingivalis, *T. denticola* and *B. forsythus* was designated as the "red complex" and was strongly related to the severity of periodontal disease, in particular to pocket depth and bleeding on probing. This finding is in agreement with other investigators who examined the relationship between pocket depth and members of the red complex (Gmür et al. 1989; Simonson et al. 1992; Kigure et al. 1995; Haffajee et al. 1998; Ximénez-Fyvie et al. 2000b). In the study of Socransky et al. (1998), 64% of sites were found to be negative for any of these species, while 10% of sites harboured all three microorganisms.

Haffajee et al. (1998) demonstrated that some species of the red complex, (*P. gingivalis*, *T. denticola* and *B. forsythus*) were more prevalent and present in higher numbers in periodontitis subjects when compared with well-maintained elder and healthy individuals. Nevertheless, the shallow sites of the periodontitis patients harboured these organisms more frequently than similar sites in subjects in the other two periodontally healthier groups. The authors hypothesised that the detection of these pathogens in healthy sites of the diseased subjects may be due to infection originating from the infected deeper sites or as a result of the initial colonisation by these species.

1.6 Humoral immune response in periodontal disease

The role of microbial plaque in initiating periodontal disease has been demonstrated by several studies. In addition, the humoral immune response to periodontal pathogens is widely reported in the literature and a summary of these findings is attempted in this section. By studying the systemic and local antibody responses to putative periodontal pathogens, we hope to be able to detect changes from colonisation to infection or from disease to recovery such that they may provide diagnostic utility. The immune response consists of the innate response, which has no immunological memory and is not specific for any microorganism and the adaptive response, which targets and eliminates specific pathogens. The innate response represents the first line of defense and is activated in a short period of time after having no previous contact with the pathogen. In contrast, the adaptive response is specific and evolves after encountering a specific microorganism. Most of the pathogens that challenge the host are dealt with by the innate response. However, when this fails to succeed the adaptive response takes over *via* both the cellular and humoral immune responses. The humoral immune response involves antibodies that target specific microorganisms and memory cells that boost the response

Table 3. Microbial members of the "red" and "orange" complexes.

Red complex	**Orange complex** **Closely related species:**
Bacteroides forsythus	*Fusobacterium nucleatum ss nucleatum* *Fusobacterium nucleatum ss polymorphum* *Fusobacterium nucleatum ss vincentii*
Porphyromonas gingivalis	*Fusobacterium periodonticum*
Treponema denticola	*Prevotella intermedia*
	Prevotella nigrescens
	Peptostreptococcus micros
	Associated species:
	Eubacterium nodatum
	Campylobacter rectus
	Campylobacter showae
	Streptococcus constellatus
	Campylobacter gracilis

Microbial species that comprise the red and orange complexes, as described by Socransky et al. (1998).

to infection with the same pathogen. Both the antigen-binding domain and the constant region of the immunoglobulins perform a variety of functions that protect the host from oral diseases. Binding of the antibody to the antigen can kill bacteria or viruses and reduce toxins. The constant regions of the antibodies can opsonise pathogens for increased phagocytosis, activate complement, cause chemotaxis of phagocytes, inactivate viruses and cause lysis of some bacteria. However, despite the protective role of the antibody responses against infection, microorganisms often trigger defense mechanisms that eventually harm the host.

The humoral immune response to periodontal pathogens has a systemic and a local aspect, producing antibodies found in serum and GCF, respectively. Three models of the host response to periodontopathogens have been proposed that highlight the relationship between local and systemic immune responses (Kinane et al. 1999b). The antigens in dental plaque challenge the host as they pass through the junctional epithelium and spread within the underlying tissues. They are then processed by cells of the innate response and antigen presenting cells travel *via* lymphatics and reach the lymph nodes, where the adaptive immune response is activated and B-cells are stimulated. **Model 1.** Stimulated B-cells produce plasma cells, which then secrete antibodies. Antibodies leave the lymph nodes and travel *via* blood vessels back to gingiva where they are excreted into the gingival crevice *via* the dilated blood vessels. In the gingival crevice, antibodies cause aggregation, precipitation, detoxification, opsonization, phagocytosis and kill the pathogens. **Model 2.** Activated B-cells, T-cells and plasma cells leave the lymph nodes and home to the local gingival tissues *via* the blood vessels. Chemokines and microbial molecules recruit these cell types to the inflamed connective tissue where plasma cells produce antibodies. Antibodies are then exuded in the GCF to encounter the pathogens. **Model 3.** Precursor plasma cells leave from the lymph nodes and travel *via* blood vessels and home and lodge in the gingival tissues, from where they proliferate and produce specific antibodies to the plaque antigens. Specific antibodies are then excreted in the gingival crevice where they encounter periodontal pathogens and provide protection against them.

It has been demonstrated that the infiltrating lymphocytes in the inflamed periodontal tissues (gingiva and granulation tissue) were more likely to originate through selective homing rather than local proliferation (Takahashi et al. 1996; Koulouri et al. 1999).

Therefore, these data point towards model two as the most likely mechanism of the immune response involved in the local periodontitis lesion.

1.6.1 Antibody function

Antibody avidity is a measure of the net binding strength between multivalent antigens and polyclonal antibodies. It reflects the functional activity of the antibodies and as a consequence, the maturation of the immune system. It has been shown that antibodies of high avidity are protective against disease and the evidence for this statement is summarised in this section.

In a cross-sectional study, O'Dell and Ebersole (1995) examined antibody avidity in: 1) non-periodontally affected subjects, 2) *A. actinomycetemcomitans*-infected chronic periodontitis patients, 3) *A. actinomycetemcomitans*-infected localised aggressive periodontitis patients, 4) chronic periodontitis patients with various antibody patterns and disease presentation. It was shown that although there were significantly higher antibody levels in *A. actinomycetemcomitans* positive patients than in chronic periodontitis patients and healthy subjects, there were no significant differences in the antibody avidity among them. A trend for avidity to correlate with the number of disease active sites (sites with attachment loss) and pocket depth was noted for the patient groups. These observations imply that there is a relationship between active disease and the presence of antibodies of high avidity. Therefore, antibody avidity to *A. actinomycetemcomitans* could help to explain the relationship between the host response and chronic infection with this pathogen.

Similarly, Mooney et al. (1993) showed that IgG antibody avidities to *P. gingivalis* and *A. actinomycetemcomitans* were not significantly higher in periodontitis patients than in control subjects. However, this study did not examine whether these subjects were infected with the homologous organisms. The authors suggested that antibody avidity could have prognostic value, by demonstrating significantly higher IgM antibody avidity to *P. gingivalis* in patients who did not experience attachment loss during a three month study period than in those who did.

In addition, Holbrook et al. (1996) found that antibody avidity to suspected periodontopathogens was higher in refractory patients than in age- and sex-matched

control subjects. IgG, IgA and IgM avidities to *P. gingivalis* and IgG to *A. actinomycetemcomitans* were higher in patients than in controls, probably as a result of the regular recalls for hygiene therapy before baseline, which may have boosted their immune response.

Lopatin et al. (1991) demonstrated that IgG antibody avidity to *P. gingivalis* was significantly elevated in chronic periodontitis patients compared to healthy individuals, while no differences existed in IgM antibodies. This could be a result of repeated antigenic exposure of periodontitis patients to *P. gingivalis* during the chronic process of disease, whereas IgM failed to reveal any differences between patient and control sera, since this class of antibody is not representative of the maturation of the immune system. Although, a significant increase in IgG antibody avidity was seen in the patient sera, avidity was extremely low when compared to the avidity levels obtained in rabbits after immunisation with *P. gingivalis*. The authors hypothesised that the reason for human antibodies of low avidity might be that when putative periodontal pathogens are present at sites, they consume the high avidity antibodies and remove them from the circulation. However, this study showed that the control subjects exhibited antibodies of lower avidity than the patients. The latter are expected to have a greater potential for antibody consumption than the former and therefore, it seems unlikely that this occurred. In addition, this potential action of antibodies should have a greater impact on the local synthesis of antibodies (in GCF) rather than the systemic (in serum). Other explanations for this could be high antigenic load (Adkinson et al. 1979), chronic exposure to the antigens (Kuriyama, 1973), or *in utero* tolerisation to bacterial antigens (Fitzgerald et al. 1988).

In contrast, Saito et al. (1993) found that periodontally healthy individuals exhibited higher antibody avidity to the fimbria antigen of *A. actinomycetemcomitans* than did chronic periodontitis patients. A statistically significant negative correlation existed between titres and avidities in healthy individuals and in *A. actinomycetemcomitans* culture-positive patients, while a positive correlation was found between titres and avidities in *A. actinomycetemcomitans* culture-negative patients. These data suggest that the antibodies produced against the fimbria antigen of *A. actinomycetemcomitans* may provide protection against infection with this organism.

Chen et al. (1991) analysed the sera of generalised aggressive periodontitis patients and healthy subjects for IgG antibodies reactive with three antigens of *P. gingivalis*. It was demonstrated that antibody avidity to all antigen preparations to *P. gingivalis* were lower in the sera of patients than in the control sera. This finding indicates that individuals who are resistant to generalised aggressive periodontitis are able to elicit an immune response that produces lower antibody titres, but antibodies of higher avidity. In addition, this study revealed a statistically significant positive correlation between antibody titres to whole-cell antigens and both mean pocket depth and bone loss, and a significant negative correlation between antibody avidity to whole-cell antigens and mean bone loss and pocket depth. These findings implied that as avidity increases the severity of periodontal disease decreases. Data from Whitney et al. (1992) demonstrated that in generalised aggressive periodontitis patients, IgG antibodies were of low avidity. Antibody avidity was higher in the seropositive healthy subjects and it was not different between seropositive and seronegative patients. However, no correlation existed between antibody avidity and severity of periodontal disease with respect to pocket depth or radiographic bone loss in the patient group.

Studies have shown that elevated antibody avidity in healthy controls may reflect chronic exposure to putative periodontal pathogens, while other investigations failed to show significant differences in antibody avidity between healthy and periodontitis subjects. Nevertheless, the majority of the literature consistently points to the protective role of high avidity antibodies from infection, by demonstrating antibodies of lower avidity in the sera of periodontitis patients than in the healthy controls.

1.6.2 Systemic antibody responses

Increasing IgG antibody levels to *A. actinomycetemcomitans* have been shown to correlate with an increase in the number of infected sites (O'Dell and Ebersole, 1995). Saito et al. (1993) demonstrated that patients with no cultivable *A. actinomycetemcomitans* exhibited higher serum IgG titres to the fimbria antigen than did those from whom *A. actinomycetemcomitans* was cultivated. The authors speculated that antibodies against the fimbria antigen contributed to the clearing of *A. actinomycetemcomitans* from the periodontal pockets. Mooney et al. (1993) showed that IgG and IgA antibody titres to *P. gingivalis* were higher in periodontitis patients than healthy controls, while Holbrook et al. (1996) found that only IgA antibody titres to

P. gingivalis were higher in refractory patients than in controls. In another study, it was demonstrated that mean IgG antibody titres to *P. gingivalis* were significantly elevated in the sera of patients with chronic periodontitis (Lopatin et al. 1991). However, no differences were found in IgM antibody titres. Since the antibodies to *P. gingivalis* were of low avidity, it appeared that the presence of elevated antibody titres may have reflected the presence of increased levels of microorganisms in the pocket and, thus the ineffectiveness of the immune response to clear the periodontal pathogens.

In another study, 36 patients with generalised aggressive periodontitis were recruited, of whom 12 showed antibody titres to *P. gingivalis* whole-cell antigens at least two-fold greater than the median of the healthy individuals, and were designated as seropositive, while the remaining 24 patients were seronegative (Chen et al. 1991). A significant negative correlation was found between antibody titres and avidities for the seropositive, but not for the seronegative patients, indicating that while the titres were high for the seropositive subjects, the avidities were low. During the course of disease one-third of the patients produced high levels of antibodies, but these were of low avidity to *P. gingivalis*, which one may consider unlikely to protect against infection. The remaining two-thirds of patients did not seem to raise a biologically functional immune response, as levels and avidities of antibodies were lower than those of the healthy controls. In addition, mean percentage bone loss was significantly greater for the seropositive than the seronegative patients. This observation raises many questions, but it is clear that elevated antibody titres and low avidities may not be protective and indicate disease and pathogen exposure. In addition, it is possible that seropositive patients could have been exposed to disease for a longer period of time, or that disease progression in this group might have been more rapid.

It has been shown that serum IgG levels to *A. actinomycetemcomitans* strain Y4 were associated with localised aggressive periodontitis (Ebersole et al. 1982b). Serum and salivary IgA and serum IgE antibody levels were related to generalised and localised aggressive periodontitis. These responses were specific to the Y4 strain. The authors suggested that monitoring the antibody responses may help us diagnose and differentiate different types of periodontal disease. Another study reported low levels of antibodies to *P. gingivalis* in periodontally healthy children and adults and higher levels of IgG antibodies in sera of patients with chronic and generalised aggressive periodontitis than

in adult healthy controls (Mouton et al. 1981). However in this study, half of the diseased patients showed low antibody levels to *P. gingivalis* and the authors speculated that this could be a result of: 1) pathogenic bacteria other than *P. gingivalis* that predominate in the periodontal pocket and elicit an immune response, 2) fluctuations in the subgingival microflora leading to periods with low numbers of *P. gingivalis* and specific antibodies, 3) infecting organisms causing immunosuppression and production of low levels of antibodies. Ebersole et al. (1986) found elevated serum IgG antibody titres to *P. gingivalis* in advanced chronic periodontitis patients, which is consistent with data from the study by Mouton et al. (1981). In addition, increased IgG antibody levels to *P. intermedia* were shown in the advanced periodontitis patients and elevated systemic antibody responses to *Bacteroides* species were more frequently found in periodontitis patients, possibly reflecting colonisation by these organisms.

In contrast, Whitney et al. (1992) assessed the immunological parameters of generalised aggressive periodontitis patients and compared them with those of healthy individuals. It was found that a proportion of healthy controls had significantly elevated serum IgG antibody levels to *P. gingivalis*. This finding might be due to the fact that these subjects were exposed to the bacteria at some time and had elicited an immune response to the antigens but did not have detectable clinical signs of disease, or that the antibodies of high avidity found in these seropositive controls protected them from clinical disease. A significant positive correlation was found between IgG anti-*P. gingivalis* titres and severity of bone loss, but not pocket depth in the seropositive patients, indicating that the high levels of antibodies may not be protective. In contrast, another study demonstrated that more than 81% of patients with destructive periodontitis exhibited elevated antibody to at least one of the 18 microorganisms tested, but no pattern of elevated antibody responses could be directly related to the severity of attachment loss (Haffajee et al. 1988a).

IgG antibody reactivity to *A. actinomycetemcomitans* and *P. gingivalis* has been shown to be inversely related to the number of affected teeth, but did not correlate with attachment loss in patients with localised and generalised forms of aggressive periodontitis (Gunsolley et al. 1987). This finding indicates that the failure to mount an increased antibody response to these organisms leads to more widespread periodontal disease. Other reports linked only elevated levels of anti- *P. gingivalis* IgG antibodies

to the degree of periodontal destruction in chronic periodontitis patients (Naito et al. 1985; Gmür et al. 1986). The lack of agreement in the relationship between antibody titres and clinical severity among various studies could be explained by differences in the disease severity measurements, limitations in detecting clinical changes by conventional clinical assessment techniques, high variability among patients who have different types of periodontal disease and relatively small sizes of patient groups.

IgG antibody titres to *P. gingivalis* and *B. forsythus* in chronic and aggressive periodontitis patients have been investigated (Califano et al. 1997). It was shown that antibody titres to *B. forsythus* were quite low with no significant differences between the periodontitis groups and the healthy individuals, while levels of antibody to *P. gingivalis* were significantly higher in the periodontitis patients than in the controls. Periodontitis patients and healthy controls showed the same percentage of subjects seropositive and seronegative to *B. forsythus*, whereas a higher percentage of *P. gingivalis* seropositive subjects was seen among the periodontitis patients than the controls. The low antibody response to *B. forsythus*, could be explained by the possible absence of this organism in many patients or by the inability of this organism to invade host tissues and thus provoke a substantial immune response. As the detection frequency of *B. forsythus* is relatively high in chronic periodontitis patients, and since this organism is usually found with *P. gingivalis*, the former explanation seems unlikely. The results of this report showed that *B. forsythus* elicited an antibody response in a small percentage of patients and based on these data the authors questioned the pathogenic role of *B. forsythus* in periodontal disease.

It has been shown that among patients exhibiting destructive periodontal disease, clinical characteristics, predominant cultivable microorganisms and antibody levels differed, suggesting the existence of different patterns of disease in these subjects (Haffajee et al. 1988a). Taubman et al. (1992) monitored clinical and immunological parameters of subjects with prior periodontal disease over five years. The majority of the subjects showed elevated antibody levels to 18 subgingival species and these remained fairly constant over time. Fluctuations in the antibody response were noted for only a few species. The authors speculated that elevated antibody titres could be a result of bacteraemias caused by the pathogens, increases in the antigenic burden in the gingival tissues, invasion of the pathogens in host tissues during the destructive process

or inoculation of the antigens during therapy. Alternatively, a decrease in the antibody levels may originate from therapeutic elimination of the bacteria or host-mediated reduction of the antigens.

In summary, most studies tend to agree that elevated serum antibody levels reflect exposure to periodontal pathogens, since this was a common finding in periodontitis patients. In addition, there seems to be agreement that elevated antibody levels in conjunction with low antibody avidity reflect a compromised host response to bacterial challenge and therefore a susceptibility to disease initiation or progression.

1.6.3 Site-specific antibody responses

Studies have shown that IgG, IgA and IgM antibodies are present in GCF (Brandtzaeg, 1965) and that serum immunoglobulins infiltrate the interstitial fluid in inflamed gingival tissues and are excreted in the gingival crevice (Challacombe et al. 1978). Aspects of both systemic and mucosal immune responses are involved in periodontal disease. Kinane et al. (1999b) demonstrated that the lesion near the gingival margin presented features of a mucosal immune response with predominance of IgA expressing plasma cells and the deeper granulation tissue showed elements of a systemic response with the presence of plasma cells expressing IgG and IgM antibodies. GCF antibodies are both serum derived (Ebersole et al. 1986, 1987) and produced locally by plasma cells in the diseased periodontal tissues (Ebersole et al. 1985a, 1985b). Aspects of the local immune responses, such as the association of GCF antibodies with the presence of homologous bacteria, the specificity of the local responses and the correlation between GCF antibody levels and disease activity have been investigated in several studies. Their findings are summarized in this section.

An early report revealed site-to-site variations in GCF antibody levels in the oral cavity of periodontally diseased patients (Ebersole et al. 1984). At certain sites local antibody levels exceeded those of the systemic circulation, indicating synthesis of specific local antibody in gingival tissues. Other studies by the same investigators confirmed previous findings and suggested that a local host response against microorganisms colonising different sites may be detected by monitoring antibody levels in the crevicular fluid (Ebersole et al. 1982a, 1985a). In accordance with these data, Tew et al. (1985) found a marked site-to-site variability in local antibody synthesis among different sites of similar

clinical status. Although antibody titres in GCF to most strains were lower than those in serum, local antibodies to *P. gingivalis* and *A. actinomycetemcomitans* were often markedly higher than systemic antibodies, indicating local synthesis of specific antibodies to these organisms.

Local responses seem to be at least as specific as serum responses against the test bacteria (Ebersole et al. 1984; Smith et al. 1985). The coexistence of local antibody, homologous organism and active disease suggests that GCF antibodies are the result of specific local challenges in diseased subjects (Ebersole et al. 1982a). Lally et al. (1980) investigated the specificity of the local antibody response in periodontal disease and suggested that IgG is the predominant immunoglobulin isotype produced in chronically inflamed gingival tissues. In addition to IgG, the synthesis of IgA was also demonstrated, but not that of IgM. No immunoglobulin antibody synthesis was detected in histologically normal gingival tissue or in fibrotic tissue. These results indicate that there is local antibody production in diseased gingival tissues and that this response is specific. Additional data confirmed that a significant portion of the antibody found in the gingival crevice is locally produced (Smith et al. 1985). The authors also showed that there was no evidence of IgM in the gingival homogenates. All the subjects who exhibited high GCF antibody levels also demonstrated elevated IgG antibody concentration in the corresponding gingival homogenates, but antibodies in the GCF were at lower levels than those in the gingival homogenates. This may have been due to consumption of local antibodies by the organisms present in the pockets.

It has been suggested that serum antibody levels that exceed certain thresholds indicate an increased likelihood of disease activity, which takes place at some site in the oral cavity (Taubman et al. 1992). Therefore, it may be beneficial to measure the antibody levels to that species at the local sites, so that areas of active disease can be detected. Johnson et al. (1993) investigated the relationship between GCF antibody levels and local disease status of patients with generalised aggressive periodontitis. It was demonstrated that GCF volume and specific local antibody levels were positively correlated with probing depth, indicating that deeper pockets gave rise to more GCF and to higher levels of antibody to *P. gingivalis*. Results from previous studies are consistent with this finding (Naito et al. 1984; Murray et al. 1989). However, other

studies have failed to correlate clinical disease status with elevated anti-*P. gingivalis* antibody levels in GCF (Tew et al. 1985; Baranowska et al. 1989).

GCF levels of IgG antibody have been shown to be lower in deep periodontal pockets than in gingivitis sites in the same patient (Mooney and Kinane, 1997). Increased consumption of locally produced antibody in periodontitis sites may be responsible for this difference. Periodontitis sites had lower levels of inflammation, deeper pockets and greater GCF volumes than gingivitis sites. Nevertheless, if levels of GCF antibody were related to volume collected, then higher median levels would be expected in periodontitis sites than gingivitis sites, but in that study antibody levels were expressed as amounts per 30-second sample. Another report from the same laboratory demonstrated lower antibody levels to *P. gingivalis* at sites with deeper pocket depth (pocket depth $\geq$ 4 mm) and greater gingival index (GI $\geq$ 3) in a group of chronic periodontitis patients (Kinane et al. 1993). Moreover, GCF IgG antibody levels against *A. actinomycetemcomitans* did not differ between sites with different pocket depths and gingival indices. However, it should be borne in mind that the diseased subjects in this study were maintenance patients who had received extensive treatment in the past and were therefore not strictly comparable with other groups of periodontitis patients or with untreated patients. These findings highlight the protective role of the local immune response and suggest that a failure of local antibody production or reduced amounts of GCF antibodies due to consumption within the pocket may reflect or contribute to the change from a gingivitis to a periodontitis lesion. Having said this, however, the increase in plasma cell density in periodontitis lesions is a significant difference from gingivitis lesions and more production of antibodies would be expected.

1.6.4 Correlation of antibody titres with the presence of homologous subgingival microorganisms

Several studies investigated the relationship between serum antibody titres against pathogens and the subgingival detection of the homologous microorganisms. It was found that serum antibody levels reflect the subgingival colonisation and infection with periodontal pathogens. This could be fundamental in the early diagnosis and prevention of subjects suffering from episodes of active disease.

Kojima et al. (1997) demonstrated that serum anti-*P. gingivalis* IgG antibody titres correlated positively with the detection frequency of *P. gingivalis* and that the antibody titres also correlated with the amount of *P. gingivalis* detected subgingivally. Although higher levels of *P. gingivalis* were seen in aggressive periodontitis than chronic periodontitis patients, no significant differences in the antibody levels existed between the two types of periodontal disease. However despite this, the functional properties of antibodies may vary between the different forms of periodontal disease. In addition, it has been shown that serum IgG antibody levels to *P. gingivalis* were positively correlated with the presence of *P. gingivalis* subgingivally but this did not apply to the other bacteria tested, including *A. actinomycetemcomitans* and *P. intermedia* (Nakagawa et al. 1990). Choi et al. (1990) found a close relationship between elevated serum antibody responses and homologous infection with *P. gingivalis* in recurrent sites, implying that this species might be important in the pathogenesis of the early stages of recurrent periodontal disease.

Similarly, another study reported a strong correlation between elevated serum and local antibody levels and subgingival colonisation of pathogens (Ebersole et al. 1982a). In a later study, the same authors attempted to determine whether elevated serum antibody levels to at least one species from a range of 18 oral microorganisms reflected homologous bacterial colonisation at active disease sites (Ebersole et al. 1987). The results of this study demonstrated that the microorganism to which the subjects exhibited elevated serum antibody responses was detected in nearly 55% of the disease active sites, while only 18% of the inactive sites harboured this microorganism. These results suggest that elevated antibody levels exist as a response to bacterial infection at disease active sites. Another study investigated the antibody responses in a distinct subset of periodontitis patients infected with *A. actinomycetemcomitans* (Ebersole et al. 1995). Active disease patients exhibited a significantly greater frequency of infected sites, as well as a significant elevation in the proportions of *A. actinomycetemcomitans.* The IgG antibody levels were significantly elevated in subjects with active disease, whereas IgA antibody titres were low in a number of the disease active patients. Plaque samples taken from active sites showed an increase in *A. actinomycetemcomitans* that occurred between two and six months prior to the identification of disease activity. Approximately 70% of the active disease group showed an increase in IgG antibody level between two and four months prior to disease activity. In an earlier study by the

same authors, the effect of root planing on the humoral immune response to suspected periodontal pathogens was assessed and the microbial species to which the elevated antibody responses were targetted, were detected in the subgingival plaque of patients (Ebersole et al. 1985c).

However, other investigators failed to associate high serum antibody levels with bacteria that were found subgingivally in a large family with a high prevalence of aggressive periodontitis (Williams et al. 1985). Conversely, putative periodontal pathogens of relatively large proportions subgingivally were found in the absence of elevated serum antibody titres. It appears that the bacterial colonisation does not necessarily lead to infection and production of protective levels of antibodies. This coincides with findings of Magnusson et al. (1991), who suggested that the failure to detect the homologous microorganisms subgingivally in subjects with refractory periodontal disease could be due to the fact that the microorganisms had been successfully eradicated by the intense therapy although, the persistence of disease would not support this conclusion.

Similarly, it has been suggested that the lack of association between antibody levels and the counts of homologous bacteria in subgingival plaques could be explained as follows: either the tested bacteria were eliminated by the host response or they were not pathogenic (Haffajee et al. 1995). However, the authors did not consider the possibility that the organisms tested may not elicit a host response with the production of specific antibodies (i.e. they were not so immunogenic), as an explanation for the lack of relation between antibody levels and homologous presence of bacteria. The authors suggested that the combination of high antibody titres to a species and the high prevalence of that species might be indicative of disease activity and progression. In addition, Whitney et al. (1992) failed to find a significant relationship between the presence of *P. gingivalis* and antibody titres of the IgG subclass or total IgG in generalised aggressive periodontitis patients, implying that some subjects in this group of patients did not respond to homologous infection by producing high levels of antibodies.

Conflicting data exist on whether elevated antibody levels reflect infection with the homologous organisms, since cross-reactions are common between gram-negative bacilli from both the oral and enteric environment. Several studies have however correlated antibody levels with the detection of specific organisms in periodontal

pockets. Possible explanations for lack of agreement among studies may be that certain species are less pathogenic or less immunogenic (able to elicit a substantial immune response) than others, or that these species are eliminated or suppressed by the host response. Other factors that may contribute to these inconsistent findings are variations in the patients' clinical condition and in the disease duration within the patients.

1.7 Untreated periodontal sites or other intra-oral niches constitute a reservoir of infection for periodontal pathogens

1.7.1 One-stage full-mouth disinfection

Non-surgical periodontal treatment typically comprises several consecutive sessions of scaling and root planing *per* quadrant or sextant at one or two-weekly intervals. Quirynen et al. (1995) introduced the one-stage full-mouth disinfection programme where they compared the clinical and microbiological effects of this treatment strategy with the more typical treatment of quadrant scaling and root planing at two-weekly intervals. The rationale behind this treatment was to prevent re-infection of the treated sites from the remaining untreated pockets and from other intra oral niches. The same investigators conducted a series of clinical trials between 1995 and 2000 and an attempt is made to give the background of the one-stage full-mouth disinfection treatment in this section.

The clinical design of the pilot study of Quirynen et al. (1995) was as follows: the control group (five subjects) received quadrant scaling and root planing at two-weekly intervals with no use of antiseptics while the test group (five subjects) received full-mouth scaling and root planing within 24 hours, together with repeated application of chlorhexidine to all intra oral niches. Patients in the test group were treated in two visits on consecutive days under local anaesthesia (i.e. teeth in the lower arch were consistently root planed in an afternoon session and the upper arch was treated the following morning). Shortly after root planing of each jaw, the following measures were applied to achieve optimal intra-oral disinfection: 1) the dorsum of the tongue was brushed with chlorhexidine 1% gel for 1 min (by the patients), 2) the oral cavity and tonsils were rinsed twice with chlorhexidine 0.2% solution for 1 min (the last 10 sec patients had to gargle), 3) the periodontal pockets were irrigated (three times within 10 min) with chlorhexidine 1% gel (using a syringe with a blunt needle) and were repeatedly irrigated at day eight. In addition, patients were advised to rinse twice daily

for 1 min with a 0.2% chlorhexidine mouthwash for the first two weeks after treatment. Participants were asked to complete a questionnaire after scaling in order to record any adverse effects after scaling such as increases in body temperature, pain score on a scale between zero and ten and number of painkillers they took. In addition, occurrence of oral ulcers and labial herpes was also recorded. Clinical parameters were measured and subgingival plaque samples were collected from the first quadrant only at baseline prior to root planing and at one and two months. The results showed a significantly greater mean pocket depth reduction of 0.8 mm for the test group compared to the control group at the two-month visit, but this was noted only for deep pockets (7 to 8 mm). The test group showed higher body temperature and higher occurrence of labial herpes after treatment.

In a follow up study, the investigators examined the same group of patients as in the pilot study but over a period of eight months (Vandekerckhove et al. 1996). They found similar results with those in their previous study. At eight months, the test group had a significantly greater mean pocket depth reduction of 1.0 mm and a gain in attachment level of 1.8 mm over the control group for the deep pockets only.

Bollen et al. (1998) examined the clinical and microbiological effects of the one-stage full-mouth disinfection on 16 patients who were followed over four months. The clinical design of the study was similar to that of previous studies except that patients were instructed to rinse with chlorhexidine 0.2% solution for two months instead of two weeks post-treatment and to spray the tonsils with 0.2% chlorhexidine spray during this period. The results showed that the pocket depth reduction and microbiological findings post-treatment were more favourable than those in the pilot study (Quirynen et al. 1995). This observation emphasises the beneficial effects of the extended use of chlorhexidine on clinical and microbiological parameters. When deep pockets ($\geq$ 7 mm) were considered, the test group had an additional 1.85 mm pocket depth reduction and a 1.20 mm attachment gain over the control group at four months.

In a subsequent study, a larger group of patients (n=40) were examined over eight months to determine the clinical benefits of the one-stage full-mouth disinfection (Mongardini et al. 1999). Patients were subdivided into two groups, chronic and generalised aggressive periodontitis patients. The clinical design was similar to that of a

previous study by Bollen et al. (1998). Results showed that the test group had a greater 1.05 mm pocket depth reduction and an extra 0.90 mm attachment gain over the control group for deep pockets at eight months. These improvements were found for all subgroups (chronic periodontitis and generalised aggressive periodontitis patients and smokers), while the greatest differences were noted for the non-smoking chronic periodontitis patients.

In a more recent study, 36 subjects were followed over eight months to evaluate the role of chlorhexidine in the one-stage full-mouth disinfection treatment (Quirynen et al. 2000). The control group (n=12) received quadrant scaling and root planing at two-weekly intervals with no use of antiseptics and the test group (n=12) received the one-stage full-mouth disinfection treatment (Fdis). These two groups also took part and were treated in the previous study of Mongardini et al. (1999). A third group of 12 subjects was added and patients in this group received full-mouth root planing within 24 hours with no use of chlorhexidine (FRp). At eight months, pocket depth in deep pockets was reduced by: 3.35 mm in the Fdis group, 3.10 mm in the FRp group and 1.75 mm in the control group. The changes in attachment levels of deep pockets were as follows: 2.15 mm for the Fdis, 2.45 mm for the FRp group and 0.55 mm for the control goup. Similarly, the microbiological findings showed additional improvement for the Fdis and FRp over the control group. The differences between Fdis and FRp were negligible. The completed questionnaires revealed that on day one both the control and the FRp groups scored higher pain rating than the Fdis group, and on day two this was significantly higher for the FRp group compared to the Fdis group. This increase paralleled an increase in the intake of analgesics. Body temperature increased for patients in the test groups at the evening of day two but dropped again the following morning. Only one patient in the Fdis group had labial herpes. In conclusion, this study showed that the role of chlorhexidine was not critical in the one-stage full-mouth disinfection protocol.

1.7.2 Clinical studies that compare full-mouth versus partial-mouth treatment

Mombelli et al. (1996) tested the subgingival application of tetracycline fibres in subjects who received treatment at only two tested sites in comparison with individuals who had all their teeth treated. The tested sites had pockets deeper than 4 mm, bleeding on probing and were positive for *P. gingivalis*. Periodontal treatment consisted of

supragingival scaling, subgingival application of tetracycline fibres and subsequent scaling and root planing under local anaesthesia after removing the fibres. Only patients who received the full-mouth treatment used chlorhexidine mouth rinse for two weeks. The results of this study showed that there was a greater pocket depth reduction in the full-mouth treatment group (1.74 mm) than in the local treatment group (0.88 mm). However, there was no difference in attachment level changes between the groups. This could be explained by the fact that the general oral hygiene status in the local treatment group was compromised and that there was no adjunctive use of chrohexidine in this treatment group. Another possibility may be that the untreated sites acted as bacterial reservoirs.

Nowzari et al. (1996) investigated the amount of guided tissue regeneration and microbial contamination of expanded polytetrafluroethylene membranes after periodontal surgery on two groups of patients. Group A showed no remaining pockets $\geq$ 5 mm except for the study sites and low levels of periodontal pathogens, while group B had several deep pockets $\geq$ 5 mm and high levels of periodontal pathogens. Barrier membranes from group B had significantly more pathogens than group A. The authors were unsure whether the organisms that were recovered from the membranes were true colonisers or solely contaminants originating from other periodontal pockets in the oral cavity. It was speculated that the source of infection might originate from several areas. Pathogens may have been translocated from other deep pockets and contaminated the membranes *via* saliva, and the oral mucosa could have contaminated the membranes *via* sutures. The study site itself may have harboured these pathogens prior to membrane placement. In conclusion, this study showed that the group of patients who received periodontal osseous surgery and had no remaining pockets $\geq$ 5 mm except for the study sites exhibited the lowest levels of periodontal pathogens in the membranes and the highest clinical attachment gains.

The effects of some oral hygiene measures on *Streptococcus mutans* and interproximal dental caries in a group of children who had high salivary levels of *S. mutans* has been investigated (Axelsson et al. 1987). Patients were divided into three groups who received prophylactic measures of different intensity. In group one, patients received the most meticulous preventive treatment which consisted of a combination of professional plaque control, tongue-scraping and chlorhexidine application, while the

other two groups received less intensive prophylactic measures. Results showed that interdental and salivary *S. mutans* markedly decreased for group one, in the short-term. However, after six months and until the completion of the study no differences in *S. mutans* colonisation were detected among the three groups. These results suggest that the additional preventive measures that were provided to group one did not enhance the elimination of *S. mutans* as might have been expected. All three groups received a high standard of oral hygiene measures, and although in one of the groups the measures were more stringent, this appeared to have no clinical effect.

1.7.3 Means of intra-oral bacterial translocation

Numerous studies, in addition to the clinical trials on the one-stage full-mouth disinfection, attempted to test the hypothesis that intra-oral transmission of pathogens occurs and to define the mechanisms by which this may occur. It has been shown that putative periodontal pathogens are frequently detected in supragingival plaque deposits (Ximénez-Fyvie et al. 2000b) and there is the potential for them to spread to uninfected sites or re-colonise periodontal pockets that have been previously treated (Quirynen et al. 1999). A study by Danser et al. (1994) failed to detect certain periodontal pathogens such as *A. actinomycetemcomitans* and *P. gingivalis* in edentulous patients, suggesting that these organisms preferably colonise periodontal pockets rather than other oral niches. This is in agreement with other studies, which suggest that the primary ecological niche for *A. actinomycetemcomitans* is the periodontal pocket (Slots et al. 1980; Könönen et al. 1991). On the contrary, *P. intermedia* and other *Prevotella* species were recovered from the oral mucous membranes before and after full-mouth extractions in patients with severe periodontitis, implying that these organims may be commensal microorganims of the oral cavity (Danser et al. 1994). Translocation of microorganisms from one site to another may occur *via* the supragingival plaque deposits and also by saliva, oral hygiene aids (toothbrushes, floss, interdental brushes) and / or dental instruments (periodontal probes, syringe tips).

The potential of bacterial transmission from periodontally affected teeth to implants in partially edentulous patients was investigated by using differential phase contrast microscopy (Quirynen et al. 1996). The results of this study confirmed that this occurs and showed that higher proportions of spirochaetes and motile organisms were found around the implants when teeth and implants were present in the same jaw rather than

the antagonistic jaw. In addition, pockets around teeth and implants of the same disease severity were found to contain a similar subgingival microflora. The coexistence of *A. actinomycetemcomitans* in stimulated saliva and subgingival sites was demonstarted (Asikainen et al. 1991), and similarly, another study showed that *P. gingivalis* and *P. intermedia* were present in the saliva of untreated chronic periodontitis patients (van Winkelhoff et al. 1988). These findings imply that there is a possibility of intra-oral transmission of these putative pathogens *via* saliva.

It has been shown that *S. mutans* can be transmitted intra-orally with a dental explorer (Loesche et al. 1973, 1979) and this finding led many investigators to examine the potential of a periodontal probe as a means for intra-oral transmission of pathogens. The majority of sampled periodontal probes contained bacteria with morphologic characteristics of a pathogenic microflora (Barnett et al. 1981). High numbers of bacteria including putative periodontal pathogens were recovered from periodontal probes implying that an intra-oral translocation of the adhered bacteria might occur during probing (Papaioannou et al. 1996). A study by Preus et al. (1993) revealed that tips of syringes used for subgingival application of minocycline in periodontal pockets were contaminated with subgingival bacteria resistant to the antibiotic. These bacteria were found to survive on the syringe tips for up to eight days after the clinical procedures.

Another study showed that toothbrushes and toothpaste were infected with *S. mutans* 24 hours after usage (Svanberg, 1978). It has been suggested that although there was no clear time of toothbrush contamination, contamination might have occurred between one week and one month after use (Glass and Lare, 1986). Müller et al. (1989) examined toothbrushes from periodontitis patients harbouring high numbers of *A. actinomycetemcomitans* subgingivally to detect contamination of the toothbrushes with this organism. It was shown that immediately after toothbrushing 62% of the toothbrushes were contaminated with *A. actinomycetemcomitans* while this dropped to 50% after one hour. Significantly higher numbers of *A. actinomycetemcomitans* could be detected on the toothbrushes when the organism was present on the oral mucous membranes or in the saliva. Although there is evidence that oral hygiene aids can become contaminated with oral bacteria, it is difficult to prove whether this could result

in inoculation of microorganisms into sites currently free of these organisms (Müller et al. 1989).

In conclusion, these studies suggest that dental instruments can result in translocation of pathogens from one site to another. However, it still remains unclear whether dental procedures can cause inoculation of bacteria and thus infection of new sites. This is supported by another study which demonstrated that *A. actinomycetemcomitans* can be transmitted by periodontal probes to healthy periodontal sites in localised aggressive periodontitis patients, although the pathogen was eliminated from the recipient sites in three weeks (Christersson et al. 1985). The authors speculated that protective mechanisms such as mechanical removal, bacterial antagonism, and specific or non-specific host responses might be responsible for the elimination of the microorganism from the recipient gingival sulci.

1.7.4 The significance of supragingival plaque control in the maintenance of stable periodontal conditions and in the prevention of intra-oral bacterial transmission

Several investigators demonstrated that effective supragingival plaque control significantly affected the composition of the supragingival and subgingival microflora. Studies using darkfield microscopy showed that professional supragingival plaque control resulted in reduction of the motile organisms and spirochaetes in subgingival plaque samples (Smulow et al. 1983; Magnusson et al. 1984; Müller et al. 1986; McNabb et al. 1992; Katsanoulas et al. 1992), while culture techniques showed a reduction in the levels of bacteria including *A. actinomycetemcomitans* and *P. gingivalis* (Smulow et al. 1983; Dahlén et al. 1992; McNabb et al. 1992; Hellström et al. 1996). In addition to microbiological changes, improvement in clinical indices was also detected.

In agreement with previous studies, Ximénez-Fyvie et al. (2000a) showed that weekly professional supragingival plaque control in maintenance patients reduced counts of both supra- and subgingival species at moderately deep pockets for a period of nine months using checkerboard DNA-DNA hybridization technique. Haffajee et al. (2001a) showed that manual and powered toothbrushing resulted in significant reductions in pocket depth, plaque index and bleeding on probing in periodontitis patients that were enrolled in a maintenance programme. A follow up study by the same investigators,

demonstrated that both toothbrushing groups exhibited reduced supra- and subgingival counts and prevalence of most species examined (Haffajee et al. 2001b). Nevertheless, a clinical study showed that during the first three months of the study plaque control measures alone resulted in a limited pocket depth reduction (0.4 to 0.5 mm), despite the low plaque scores that were achieved (Badersten et al. 1984a). This improvement was primarily due to gingival recession and limited improvement in bleeding scores was also noted. These changes were inferior to the enhanced clinical outcome following scaling and root planing during the subsequent three months.

It is doubtful whether supragingival plaque control affects the levels of pathogens within deep periodontal pockets (Listgarten et al. 1978; Kho et al. 1985; Beltrami et al. 1987; Loos et al. 1988). This finding suggests that the periodontal pocket is a separate ecological niche independent from the supragingival environment and that the nutrients for the subgingival microorganisms are supplied by other members of the microflora or by the GCF. Additional data showed no differences in the patterns of subgingival bacterial re-colonisation between sites under strict supragingival plaque control and control sites (Lavanchy et al. 1987). The authors suggested that the bacteria detected in the pockets following instrumentation rather than the meticulous supragingival plaque control measures, may play a more important role in the re-establishment of the subgingival microbial flora. Supragingival plaque control on its own was not shown to be successful in halting the progression of periodontal disease, particularly at sites with initial pocket depths exceeding 6 mm (Westfelt et al. 1998). Nevertheless, a reduction in the total number of subgingival microorganisms of the sampled sites was observed regardless of baseline pocket depth. Another study showed that the clinical outcome of supragingival treatment alone was not as favourable as that observed after subgingival instrumentation (Kaldahl et al. 1996a). The authors speculated that the clinical improvements seen at the sites which were treated only supragingivally may have resulted from an alteration in the subgingival microflora or from immunological changes caused following subgingival instrumentation in other areas of the same mouth.

In contrast, two studies showed that carefully performed supragingival plaque control over a prolonged period of time significantly affected the quantity and composition of the subgingival microflora at periodontal sites including deep periodontal pockets (Dahlén et al. 1992; Hellström et al. 1996). Similarly, it has been shown that the

numbers of subgingival anaerobes at deep periodontal pockets were influenced by supragingival plaque control over 21 days of observation (Smulow et al. 1983). Lack of agreement among studies could be explained by differences in the patient populations and in the severity of disease; in the sampling frequency and sampling technique; in the frequency and nature of application of plaque control measures (professional plaque control and intensive programme of oral hygiene instruction against unsupervised self-performed plaque control). In some of the studies mentioned, root planing was performed in addition to supragingival scaling.

Using an animal experimental model, it was shown that subgingival plaque formation could be arrested in areas that were accessible to the toothbrush (Waerhaug, 1981). However, optimal plaque control could not be achieved solely by toothbrushing, particularly at the interdental areas where supragingival deposits are most likely to accumulate. Mousqués et al. (1980) found marked clinical and microbiological improvements after one-day full-mouth scaling and root planing without giving patients any oral hygiene instruction. Pocket depth improvement was maintained over the 90 days of observation. However, in the absence of plaque control, subgingival microflora returned to baseline values with spirochaetes taking as long as 42 days to re-populate the sites. Similarly, Magnusson et al. (1984) showed that in presence of supragingival plaque a subgingival microflora with a predominance of spirochaetes and motile rods was soon re-established at four to eight weeks. In another study with a similar clinical protocol, therapy consisted of a single course of scaling and root planing and no oral hygiene instructions and patients were followed over eight weeks (van Winkelhoff et al. 1987). This treatment resulted in significant probing pocket depth reduction and gain in attachment level. Nevertheless, no further clinical improvement was seen two weeks after treatment.

In conclusion, the results of the studies described above showed that optimal supragingival plaque control can inhibit the microbial re-population of subgingival pockets, which have been periodontally treated. As a result, periodontal deterioration can be arrested at a periodontally diseased site by controlling the plaque formation supragingivally, despite the presence of subgingival calculus. Nevertheless, it has been shown that in the presence of supragingival plaque deposits, it takes approximately 42 days for the subgingival microflora to shift back to pre-treatment values.

1.7.5 The role of chlorhexidine in the treatment of periodontal disease

It has been shown that following surgery, and during the healing period, professional tooth cleaning was a slightly more efficacious plaque control measure compared to chlorhexidine mouth rinsing (Westfelt et al. 1983). Despite the fact that both procedures were equally effective in supragingival plaque control, pocket depth reduction and gain in attachment levels were slightly greater in the patients who received professional plaque control post-surgery. It was suggested that this may be due to the fact that chlorhexidine more likely had no effect on the subgingival plaque formation or on the microflora at deep periodontal pockets. This is in agreement with the results of another study, in which the effect of chlorhexidine on plaque accumulation and gingival conditions in a group of young adults was investigated (Flötra et al. 1972).

In addition, there is evidence that chlorhexidine irrigation (2%) of deep pockets did not enhance the beneficial effects of meticulous scaling and root planing and oral hygiene instructions (Braatz et al. 1985; MacAlpine et al. 1985). Similarly, periodic subgingival irrigation with chlorhexidine (0.2% solution) as a single measure of treatment as well as combined with mechanical debridement in patients with moderate to severe periodontitis was found to have only limited and temporary effect on the subgingival microflora and clinical parameters in deep periodontal pockets (Wennström et al. 1987a, 1987b). It is of great interest to see that adjunctive irrigation with chlorhexidine or hydrogen peroxide did not improve healing to a greater extent than that obtained after mechanical treatment alone or in combination with saline irrigation. Additional data showed that the effects of a single professional subgingival irrigation with antimicrobial agents (0.12% chlorhexidine, 5% tetracycline) or saline did not result in a greater reduction of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*, compared to that achieved by meticulous scaling and root planing (Shiloah and Patters, 1994). In a follow up study, the adjunctive use of these antimicrobials was not shown to affect the rate of re-population of periodontal pockets ($\geq$ 5 mm) by these species (Shiloah and Patters, 1996).

In contrast, chlorhexidine 2% gel was found to significantly reduce the number of black pigmented *Bacteroides* species by 99% within 30 min of application in 5-9 mm deep periodontal pockets. However, whether this short-term effect of chlorhexidine on the

subgingival microflora is significant in the maintenance of periodontitis patients still needs clarification (Oosterwaal et al. 1991). The beneficial effect of the use of chlorhexidine (0.2% solution) in periodontitis patients who received supragingival debridement and no oral hygiene instructions over a two-month period has been demonstrated (Soh et al. 1982). Using a similar study design, Haskel et al. (1986) demonstrated that the subgingival irrigation with chlorhexidine 0.2% solution in moderate chronic periodontitis patients had a transient microbiological effect and limited clinical benefit. Another study demonstrated that 2% chlorhexidine irrigation of deep pockets ($\geq$ 6 mm) as a sole therapy was nearly as effective as scaling and root planing, in terms of clinical improvement and reduction in the levels of *P. gingivalis* (Southard et al. 1989). However, the combination of these therapies resulted in an enhanced attachment gain and a further reduction in the levels of *P. gingivalis*. It should be stressed, however, that the protocol of this study included no reviews or oral hygiene instructions throughout the monitoring period (15 weeks).

It has been demonstrated that a single subgingival irrigation with chlorhexidine (0.2% gel or solution), as the only measure of treatment in moderately deep pockets, decreased the mean percentage of spirochaetes and motile bacteria, with a concomitant increase in the mean percentage of cocci and "other" microorganisms (Lander et al. 1986). In addition, there was a decrease in the mean percentage of bleeding sites, but not in the plaque and gingival index and attachment levels. The suppression of the microorganisms remained up to four weeks. In a further study, basic oral hygiene instructions were given and the periodontal pockets ($\geq$ 4 mm) were treated with one session of scaling and root planing and the adjunctive use of 0.2% chlorhexidine solution or metronidazole (Khoo and Newman, 1983). It was demonstrated that both antimicrobial agents yielded greater beneficial changes in subgingival microflora than mechanical control alone which lasted up to 84 days. Soskolne et al. (1997) tested a degradable subgingivally placed drug delivery system containing 2.5 mg chlorhexidine as an adjunct to scaling and root planing in chronic periodontitis patients with pockets of 5 to 8 mm depth. The supplementary use of chlorhexidine to scaling and root planing provided a greater pocket depth reduction at three and six months. However, it must be emphasised that during this clinical trial no oral hygiene instructions were given.

In conclusion, differences in the plaque control levels during the experimental period may have contributed to the conflicting results across the studies. In some clinical trials there was a lack of meticulous plaque control and in others no scaling and root planing was performed prior to the use of chlorhexidine. Therefore, the studies comparing test and control subjects, both of which had inadequate plaque control, gave misleadingly promising results for the adjunctive use of chlorhexidine.

1.8 Efficacy of non surgical periodontal therapy

The purpose of scaling and root planing is to: 1) eliminate or suppress the pathogens, 2) shift the microflora to one that is less pathogenic to the host, 3) alter the local environment to one which does not favour plaque accumulation and multiplication of organisms, and 4) develop a more competent host immune response. The effects of periodontal therapy on the clinical, immunological and microbiological parameters are summarised in the following sections.

1.8.1 Effect of scaling and root planing on clinical parameters

Numerous investigations have documented the efficacy of scaling and root planing in the treatment of periodontal disease. Studies have shown that the amount of pocket depth reduction after treatment is related directly to the initial pocket depth with the greatest reduction for the deepest pockets (Knowles et al. 1979; Pihlstrom et al. 1983; Ramfjord et al. 1987). In addition, a relationship is apparent between attachment changes and initial pocket depth. Comparisons of before and after treatment data are a crucial element of analyses of therapeutic studies. However, it should be taken into consideration that statistical errors may arise if these data are used in regression models as the baseline and endpoint data are not truly independent and are subject to bias due to mathematical coupling. Difficulties or biases may also arise due to the phenomenon of regression towards the mean. Therefore, regressing / correlating post-treatment changes of variables with their baseline values should be treated with care.

In a thorough review of the literature, the mean probing pocket reduction and mean attachment gain following scaling and root planing were determined for shallow, deep and moderately deep sites (Cobb, 1996). It was demonstrated that shallow pockets (1-4 mm) and moderately deep sites (4-7 mm) had a mean pocket reduction of 0.03 mm and 1.29 mm, respectively, while deep sites (> 6 mm) showed the greatest pocket reduction

of 2.16 mm. The mean attachment gain for shallow, moderately deep and deep sites was –0.34 mm, 0.55 mm and 1.19 mm, respectively. That study also showed a mean reduction in bleeding on probing of 57% post-therapy. Lindhe et al. (1982a) demonstrated that in a well controlled oral hygiene regimen, meticulous scaling and root planing was equally effective when used alone as in combination with the modified Widman's procedure in the treatment of advanced periodontitis. A high frequency of probing depth of less than 4 mm was seen following both treatment modalities and the authors speculated that the gain of attachment after treatment reflected a reduction in the degree of gingival inflammation rather than a true gain of connective tissue attachment. The clinical improvements after mechanical debridement remained unchanged during a maintenance period of 18 months. During this period recurrence occurred but it was a rare finding, and when it did develop it was considered to be related to either ineffective prophylactic measures or inadequate debridement during active treatment (Waerhaug, 1978).

Isidor et al. (1984) demonstrated that periodontal therapy resulted in significant clinical improvements in patients with advanced periodontal disease. Root planing resulted in marked pocket depth reduction, although more shallow pockets were obtained after modified Widman flap and reverse bevel flap surgery. Gain in attachment was obtained by all treatments, but root planing resulted in slighty more gain in attachment than the other two procedures. Data from another study showed that non-surgical periodontal treatment of patients with severely advanced periodontitis resulted in a marked clinical improvement in pockets of moderate depth, as well as pockets greater than 12 mm deep (Badersten et al. 1984a). Initially, 305 sites demonstrated pocket depth $\geq$ 7 mm and at the 24-month examination this number was reduced to 43. Clinical indices continued to improve during the six and nine months following start of instrumentation. This finding disagrees with the results of a previous study by the same investigators, where clinical improvement reached a plateau at three to four months after initiation of treatment (Badersten et al. 1981). This could be due to the fact that patients with less advanced disease were examined in the earlier study. Another study by the same investigators demonstrated marked clinical improvement in patients with severely advanced periodontitis (Badersten et al. 1984b). Mean pocket depth was reduced from 5.5-5.9 mm to 3.5-3.9 mm and bleeding scores from 80-90% to 15-20% after treatment. Clinical improvement reached a peak level at six to nine months after the start of

instrumentation. Data from this study suggested that a single instrumentation was equally effective as repeated episodes of instrumentation.

1.8.2 Effect of scaling and root planing on the systemic and site-specific antibody responses

Many studies have sought to determine the changes in antibody titres to putative periodontal pathogens following treatment. These changes can provide criteria on which to evaluate periodontal treatment and prognosis.

Quirynen et al. (2000) observed that seven out of 11 patients, whose body temperature rose above 37°C after the second day of the one-stage full-mouth scaling and root planing, with or without the use of chlorhexidine treatment, had an overall pocket depth reduction exceeding 3.5 mm, whereas this was noted for only four of the remaining 13 patients that did not have an increase in temperature. The observation that patients with a rise in body temperature the evening after the second day of the full-mouth treatment had the more impressive clinical improvements was considered by the authors to be due to an increased immunological reaction. However, this was a hypothesis only, since the investigators did not investigate the host immune response following this treatment.

Naito et al. (1985) reported a significant reduction in serum antibody titres to *P. gingivalis*, but not in antibody titres to other species such as *A. actinomycetemcomitans*, *P. intermedia* at 12 to 14 months after initiation of treatment. This is in agreement with the findings of another study (Tolo et al. 1982). In this study, a serum sample was collected one year after treatment and the results showed that in seven out of 12 patients the anti-*P. gingivalis* antibody levels decreased by 25% post-treatment. Except for *P. gingivalis*, most antibody levels were within the normal range before and after treatment. Pre- and post-treatment antibody titres to periodontopathic bacteria by ELISA in sera of periodontitis patients have been examined (Horibe et al. 1995). A reduction in serum antibody titres to *P. gingivalis* and *P. intermedia* at least two months (6.9 months on average) after the completion of active treatment was demonstrated. In a longitudinal study, Aukhil et al. (1988) demonstrated two distinct types of antibody response to *P. gingivalis* post-treatment. The first was, a significant reduction in the antibody titres to *P. gingivalis* during the early stages post-therapy, i.e., the end of the hygiene phase (two months after baseline approximately). The second was a significant

reduction in the antibody levels to this pathogen at the end of the maintenance phase, i.e., two years after the end of the hygiene phase. The authors concluded that periodontal treatment reduced the antigenic challenge in the periodontium and consequently the antibody titres to these antigens. Local and systemic antibody levels to *P. gingivalis* in patients with treated chronic periodontitis, untreated chronic periodontitis and gingivitis have been evaluated (Murray et al. 1989). Antibody levels decreased 12 to 16 months after treatment in the treated group, whereas significantly higher antibody levels to *P. gingivalis* were seen in the untreated periodontitis patients compared to the treated and gingivitis subjects.

Johnson et al. (1993) found that antibody titres to *P. gingivalis* in GCF decreased significantly as the clinical disease status improved post-treatment, 3 to 12 months after the completion of scaling and root planing. In a longitudinal study, a serological dichotomy to *P. gingivalis* in chronic periodontitis patients was observed (Mouton et al. 1987). The first group was characterised by pre-treatment antibody levels similar to those of healthy individuals, while the second group showed elevated levels of antibodies, indicating infection with *P. gingivalis*. Treatment resulted in a progressive reduction in IgG antibody titres to 55% of the pretreatment levels five to seven months after treatment, reaching 41% one year post-therapy for the second subgroup of patients, while the low-titre patients maintained their low antibody levels following treatment. No peak antibody levels were observed in the immediate post-treatment period, suggesting that scaling and root planing may not induce active immunisation with *P. gingivalis*. In addition, no cyclic pattern of antibody levels was observed throughout the monitoring period that would reflect the series of exacerbation and remission of disease activity, as previously described (Socransky et al. 1984). The reduction of antibody levels was attributed to the elimination of bacterial load by the periodontal treatment.

However, the previously reported findings are not consistent with the findings of Ebersole et al. (1985c), who demonstrated that scaling and root planing elicited a significant increase in circulating antibody levels to various pathogens, due to inoculation of bacteria in the host tissues. Peak levels in responses were observed two to four months after scaling and levels returned to pre-scaling levels eight to 12 months post-treatment. In addition, a significant relationship was found between increased antibody levels post-treatment and pre-existing elevated antibody titres in the patients.

In conclusion, this study supports the concept that scaling can be an effective means of active immunisation against periodontopathogens. Sjöström et al. (1994) determined the pre- and post-treatment IgG antibody titres to *A. actinomycetemcomitans* in the sera of generalised aggressive periodontitis patients. The investigators observed that antibody titres and polymorphonuclear function as assessed by polymorphonuclear leukocyte chemiluminescence assay (CL) increased significantly after treatment, whereas pocket depths decreased. When they classified the patients as either seropositive (baseline IgG titres greater than twice the median of control subjects) or seronegative, they observed that median antibody titres and CL values increased for the seronegative group at six and 12 months after the beginning of the treatment, while an increase was seen only in the antibody levels for the seropositive patients at 12 months. The increase in antibody titres may have resulted from repeated scaling and root planing treatments over an extended period of time. The authors concluded that a humoral immune response induced by scaling and root planing can be a major factor in the clinical improvement seen after therapy.

It has been demonstrated that periodontal therapy affects the quality of the immune response to suspected periodontopathogens and that this effect is depended on the initial serostatus (Mooney et al. 1995). In this study, initially seropositive patients demonstrated a significant increase in IgG avidity to *P. gingivalis*, whereas seronegative patients showed no change in IgG avidity, but had a slight increase in IgG and IgA antibody titres. No significant changes were seen in antibody avidity to *A. actinomycetemcomitans* post-treatment, and IgG, IgA and IgM antibody levels to this organism increased significantly only for the seronegative subgroup. The seropositive patients had a higher chance of clinical improvement after conventional periodontal treatment. Chen et al. (1991) investigated the humoral immune response in generalised aggressive periodontitis patients and concluded that many patients do not produce protective levels of biologically functional antibody of high avidity during the course of their natural infection, but treatment may induce the production of such antibodies. Whitney et al. (1992) investigated the humoral immune response against a whole cell homogenate of *P. gingivalis* in generalised aggressive periodontitis patients, as well as in control subjects. Their data suggest that while the avidity of anti-*P. gingivalis* IgG antibodies in seropositive patients did not significantly differ from the values for seronegative patients or seronegative control subjects, the median avidity for

seropositive control subjects was elevated compared to the other groups. This could explain the observation that although some subjects experience *P. gingivalis* colonisation, they do not develop clinical disease. In general, low titre and low avidity anti-*P. gingivalis* IgG antibodies were observed in generalised aggressive periodontitis patients, indicating a genetically determined compromise of their humoral immune response to certain types of antigens (Whitney et al. 1992).

A recent study from our laboratory showed that conventional periodontal treatment of chronic periodontitis patients resulted in significant clinical improvement and significant reduction in sites positive by polymerase chain reaction (PCR) for some of the test bacteria (*P. intermedia*, *B. forsythus* and *T. denticola*) (Darby et al. 2001). There was little change however, in the systemic and local antibody titres post-scaling, although there was a significant reduction in the IgG antibody avidity to *P. gingivalis* and *P. intermedia*. These results indicate complex interactions between the subgingival microflora and the host response and also a possible failure of the host response to produce adequate levels of biologically functional antibodies to putative periodontal pathogens.

There are great discrepancies among studies looking at the humoral immune changes following periodontal therapy. There is evidence suggesting that antibody levels decrease following treatment probably due to a reduced microbial burden, whereas other studies demonstrate increased antibody levels post-treatment which are possibly explained by inoculation of bacteria into the host tissues. These conflicting data could be attributed to differences in laboratory protocols and clinical study designs, in particular the time of testing following therapy. The majority of the literature however agrees that increased antibody avidity post-treatment indicates maturation of the immune system and therefore successful treatment. Further investigation based on standardised protocols, in terms of duration of clinical trials, sampling intervals and laboratory procedures, forms of periodontal disease and microbial species examined is required to delineate the effect of periodontal treatment on the antibody response.

1.8.3 Effect of scaling and root planing on the subgingival microbial flora

Several studies sought to determine the beneficial effects of scaling and root planing on both clinical and microbiological parameters and have consistenly reported marked

changes in the subgingival microflora and clinical indices following non-surgical instrumentation (Slots et al. 1979; Renvert et al. 1990a; Pedrazzoli et al. 1991; Shiloah and Patters, 1994; Darby et al. 2001). Nevertheless, it is unlikely that scaling and root planing can eradicate and permanently clear all the microorganisms from a site. The microbes which re-colonise a subgingival pocket post-therapy could be either residual microorganisms following incomplete instrumentation or the extension of a growing and maturing supragingival plaque. It has been shown that in the presence of supragingival plaque it takes 42 days for organisms to re-colonise a previously scaled site (Mousquès et al. 1980). In a study by Slots et al. (1979), the microbial shifts paralleled clinical improvement of patients with moderate to severe periodontitis. The kinetics of the microbial re-population over a six month period after a single course of thorough debridement and / or use of antibiotics followed three patterns: 1) rapid reduction followed by a slow return, seen for the total subgingival cell counts, including spirochaetes, 2) rapid increase in proportions of organisms followed by a slow return, observed for coccal forms and *Actinomyces viscosus*, 3) rapid reduction followed by a return which exceeds pre-treatment levels, seen for gram-negative anaerobic organisms including *Fusobacterium nucleatum*, various *Bacteroides* species, *Veillonella parvula* and gram-negative motile rods.

Scaling and root planing has been shown to have a modest effect on the composition of the subgingival microflora in chronic periodontitis patients (Haffajee et al. 1997a). Of 40 subgingival species examined, only *P. gingivalis*, *B. forsythus* and *T. denticola* significantly decreased post-treatment and none of them or any other species was undetectable on a patient-basis after therapy. For the majority of patients, most of the sites were re-colonised by pre-treatment levels of most test species three months post-therapy. Another study showed that residual pocket depth *per se* was not critical in the re-colonisation of periodontal pockets by black pigmented *Bacteroides* species and streptococci over a period of 16 weeks (Pedrazzoli et al. 1991). It has been demonstrated that scaling and root planing had a lasting suppressive effect on *P. gingivalis*, *A. actinomycetemcomitans* and *P. intermedia* for the majority of sites in patients with chronic periodontitis that received no supportive maintenance therapy over a period of 12 months (Shiloah and Patters, 1996).

The effects of modified Widman flap surgery and systemic tetracycline on the clinical and microbiological parameters at sites with evidence of active periodontal destruction have been examined (Haffajee et al. 1988b). Post-treatment sites with loss of attachment had high levels of *A. actinomycetemcomitans*, *B. forsythus*, *P. gingivalis*, *P. intermedia*, *Peptostreptococcus micros*, *Streptococcus intermedius* and *Campylobacter rectus* while the sites with no change or gain in attachment level had high levels of *Actinomyces* species, *Capnocytophaga ochracea*, *Streptococcus mitis*, *Streptococcus sanguis* and *V. parvula*. Following treatment, *A. actinomycetemcomitans* and *C. rectus* were less frequently detected, but the black pigmented *Bacteroides* had the same detection frequency as before treatment, although their mean levels decreased. The authors suggested three possible effects of periodontal therapy on the subgingival microflora: 1) pathogens remain at the same levels or increase after treatment, resulting in a compromised clinical outcome; 2) elimination of all pathogens and establishment of a microbiota which is antagonistic to their return; 3) suppression of the pathogens and an increase in the proportions of beneficial species to the host. The latter treatment outcome would require frequent maintenance procedures in order to maintain this health compatible microflora.

Other investigators have shown that in the majority of chronic periodontitis patients the detection frequency of *A. actinomycetemcomitans* decreased significantly after conservative periodontal treatment (Mombelli et al. 1994a). However, in three of ten subjects there was a high detection of *A. actinomycetemcomitans* and many of the sites harboured high levels of this organism. After treatment, the highest chance of detecting this organism was in residual pockets of approximately 5 mm. In another study by the same investigators *A. actinomycetemcomitans* was detected in all patients after treatment, with individual detection frequencies ranging from 2% to 72% (Mombelli et al. 1994b). Post-treatment, the presence of this pathogen was significantly correlated with its detection frequency before treatment and the baseline mean probing depth. There was also a significant relationship between post-treatment levels of *A. actinomycetemcomitans* and probing depth reduction and attachment gain. The authors concluded that detection of *A. actinomycetemcomitans* in multiple sites before therapy indicates a higher chance of persistence for this organism after treatment, and this might be of more importance than the mere presence or absence of this pathogen at a site.

It has been reported that in pockets of localised aggressive periodontitis patients, scaling and root planing alone could not suppress *A. actinomycetemcomitans* (Kornman and Robertson, 1985). In addition, Renvert et al. (1990a) showed that in advanced periodontitis patients (28-54 years of age), debridement resulted in significant reduction of total viable counts of microorganisms including *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*. Only proportions of *P. gingivalis* and *P. intermedia* decreased after treatment while *A. actinomycetemcomitans* tended to increase as a proportion of the viable flora six months post-treatment. Of the 38 sites that harboured *A. actinomycetemcomitans* at baseline, 28 sites were still positive for this organism after treatment, while *P. gingivalis* was present in 15 of the 52 initially positive sites after therapy. Subgingival persistence of *A. actinomycetemcomitans* appeared to be related to a compromised clinical outcome. In a follow up study, those patients who possessed at least two separately located sites harbouring *A. actinomycetemcomitans*, received further treatment which consisted of either repeated debridement or surgical excision of the gingival tissue (Renvert et al. 1990b). Neither treatment was more effective in eliminating *A. actinomycetemcomitans* than initial debridement, since this organism persisted in the majority of infected sites. The authors speculated that the difficulties in eliminating *A. actinomycetemcomitans* could be because this species resides in the gingival tissues, or could be due to a re-infection originating from other infected areas in the oral cavity. The latter explanation seemed to be more likely.

Moreover, a study by Pedrazzoli et al. (1992) showed that scaling and root planing resulted in significantly increased proportions of *A. actinomycetemcomitans* at the 2-, 12-, and 24-week examinations. The authors speculated that this could result from a relatively higher sensitivity of the other subgingival microflora to the mechanical debridement. Slots et al. (1986), showed that refractory periodontitis patients yielded predominately *A. actinomycetemcomitans*. The results reported in these studies show that *A. actinomycetemcomitans* seems to be difficult to eradicate from periodontitis lesions, possibly due to the fact that this organism can invade the gingival tissues (Saglie et al. 1982; Carranza et al. 1983). Combined mechanical debridement and use of antimicrobial therapy succeeded in suppressing *A. actinomycetemcomitans* over a long period of time, and this paralleled a further clinical improvement for up to 24 months after active treatment (Pavicic et al. 1994).

Renvert et al. (1996) treated periodontitis patients with the intention of suppressing *A. actinomycetemcomitans* and *P. gingivalis* to below detection levels and *P. intermedia* to below 5% of the cultivable subgingival microflora. Treatment to eliminate the bacteria continued for three years and the total period of observation was five years. Although the aim of the study was achieved in all sites, the indicator bacteria recurred in a number of sites over a five-year period (3% of sites for *A. actinomycetemcomitans*, 20% for *P. gingivalis* and 12% for *P. intermedia*). Patients who either lost attachment or returned to pre-treatment levels after an initial gain harboured these bacteria at a higher frequency than patients who retained their initial attachment gain.

Hafström et al. (1994) showed that, six months following treatment, abscess sites demonstrated significant improvements in pocket depth, attachment gain and bleeding on probing. Elimination of *P. gingivalis* and significant reduction in the proportion of *P. intermedia* was also demonstrated. Deep periodontal pockets also followed the same pattern. Results from another study showed a positive correlation between pocket depth reduction and decrease in *P. gingivalis* and between attachment gain and reduction in the percentage of this organism (van Winkelhoff et al. 1987). However, no correlation between these clinical parameters and other test microorganisms was seen, while an inverse relationship between *P. gingivalis* and *P. intermedia* was shown. Simonson et al. (1992) demonstrated that *T. denticola* and, to a lesser extent, *P. gingivalis* levels in successfully treated sites decreased, while non-responding sites had levels of these bacteria equal to or greater than the pre-treatment levels. In accordance with this finding are data of another study, which showed that a decrease in *T. denticola* levels was associated with successful clinical outcome following scaling and root planing (Haffajee et al. 1997b). Several suspected periodontal pathogens, including *P. gingivalis*, *T. denticola* and *B. forsythus* were found at higher levels before than after treatment in the subjects or sites that responded well to therapy.

A more recent study investigated the prevalence and counts of *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* in chronic periodontitis patients before and after treatment by use of a quantitative PCR method (Doungudomdacha et al. 2001). It was found that there was a significant decrease in the numbers of the test organisms post-scaling but none was completely eradicated, emphasising that the mere presence of a suspected pathogen may not indicate the presence of disease.

1.8.4 Microbiological findings after the one-stage full-mouth disinfection treatment

The clinical findings and the study design of the one-stage full-mouth disinfection are presented in section 1.7.1. The microbiological findings of this treatment strategy are summarised in this section. In a pilot study, Quirynen et al. (1995), collected plaque samples from the upper right quadrant of ten patients at baseline and after one and two months. At one month, differential phase-contrast microscopy revealed a significantly higher reduction in the proportion of potential periodontal pathogens (sum of spirochaetes and motiles) in the test group, whereas at two months the differences were no longer significant. The culture data showed that there were no differences in the relative proportion of aerobic colony forming units (CFUs) for both treatments. Nevertheless, when the relative proportion of potential pathogenic and beneficial species were compared, the test group showed significantly fewer pathogenic organisms at one month and more beneficial species at two months. Post-treatment, *P. gingivalis* was eliminated from the test group although this was not the case for *A. actinomycetemcomitans*, whose proportion increased following treatment, which is consistent with poor treatment findings of others (Slots et al. 1986; Pedrazzoli et al. 1992; Renvert et al. 1990a).

In a follow up study using the same protocol, samples for microbiological analysis were collected from ten patients, but patients were followed over eight months (Bollen et al. 1996). The findings from the differential phase-contrast microscopy were similar to those of the previous study, except for the eight-month observation where proportions of spirochaetes and motile rods remained lower only for the multi-rooted teeth of the test group. The culture data showed that at eight months there were no significant differences in the reduction of the anaerobic CFUs between the groups. The proportions of pathogenic species remained lower for the test group, with the exception of the four-month interval when the control group scored slightly better, but these findings were not statistically significant. The proportions of the specific species showed that no statistically significant differences existed between the two groups, although spirochaetes and *P. intermedia* remained lower in the test group up to eight months. *P. gingivalis* was eliminated in both groups over time and *A. actinomycetemcomitans* was only present in the test group at baseline, but was not detectable at eight months.

Bollen et al. (1998) examined the effect of the full-mouth disinfection on the micobiota of 16 patients with severe periodontitis over four months. The clinical design of this study included use of chlorhexidine for an extended period of time post-treatment (two months). The results showed that there was a greater reduction in the total amount of facultative and strict anaerobic CFU/ml in the test group, especially at two months with some relapse around the multi-rooted teeth of the test group at month four. The changes in CFU/ml for pathogenic species were negligible in the control group in contrast to the test group (with the exception of *A. actinomycetemcomitans*).

Quirynen et al. (1999) compared the full-mouth disinfection with the more typical treatment of four sessions of quadrant root planing on 16 patients with aggressive periodontitis and 24 patients with severe chronic periodontitis. The patients were followed over eight months and full-mouth disinfection resulted in a significantly greater reduction in spirochaetes and motiles for the chronic periodontitis patients in the test group at each follow up visit, while this was the case for the aggressive periodontitis patients only at one and two months. A significantly additional reduction in total CFU/ml and CFU/ml of black-pigmented bacteria in the test group was found for the chronic periodontitis patients at each visit, while no significant differences between the two groups were seen in the aggressive periodontitis patients at month eight. For the chronic periodontitis patients, the test group showed long-term reductions in more species of suspected periodontal pathogens (*P. intermedia*, *P. gingivalis*, *C. rectus*, *P. micros*, and *F. nucleatum*).

In a more recent study from the same laboratory, three groups of 12 patients were included and followed over a period of eight months as had been described earlier (Quirynen et al. 2000). Full-mouth root planing with and without the use of chlorhexidine resulted in a greater reduction of spirochaetes and motile organisms compared to the control group, but this was only marginally significant at four and eight months. There was a greater reduction in the total number of anaerobic organisms and black-pigmented bacteria for the test groups. A more sustained reduction in the number of CFU/ml for specific periodontal pathogens was seen for both test groups. Differences between the two test groups were negligible.

A later study compared the effects of the one-stage full-mouth disinfection and quadrant scalings on the subgingival microflora by the checkerboard DNA-DNA hybridisation technique (De Soete et al. 2001). Two groups of patients were examined, advanced chronic periodontitis patients and aggressive periodontitis patients. The results of this study showed that both treatments reduced the detection frequency and levels of the suspected pathogens for up to eight months. However, the one-stage full-mouth disinfection resulted in a greater reduction in the detection frequency and levels of the "red" and "orange" complex species (Table 3, section 1.5.6), and in the numbers of patients in whom these complexes of species were undetectable. These differences existed mainly for the chronic periodontitis patients.

1.9 Smoking and periodontal health

Numerous studies have indicated that smoking is a significant risk factor for the development of periodontal disease. It was interesting to see in an early report that none of 202 heavy smokers examined was periodontally healthy (Grossi et al. 1994). Subjects who smoked more than 30 pack-years (i.e. packs of cigarettes smoked *per* day multiplied by the number of years the subject smoked) were classified as heavy smokers. This study also showed that the severity of attachment loss was highly correlated with the number of pack-years. The odds for more severe attachment loss in smokers compared to non-smokers ranged from 2.05% for light smokers to 4.75% for heavy smokers. Similarly, a follow up study revealed that smokers had greater odds for more severe bone loss than non-smokers, ranging from 3.25% for light smokers to 7.28% for heavy smokers, and that the severity of bone loss was positively related to the number of pack-years (Grossi et al. 1995).

The prevalence of cigarette smoking was shown to be higher in both chronic periodontitis and severe generalised aggressive periodontitis patients compared with age- and race-matched periodontally healthy subjects (Schenkein et al. 1995). Another study associated the severity of periodontal condition (as determined by clinical attachment level, probing pocket depth and bone crest height) with cotinine concentrations in serum (González et al. 1996). A more recent study confirmed previous reports that smoking is a major risk for periodontitis, and suggested that smoking cessation may enhance the treatment outcome in adults with periodontitis and also reduce the risk for disease progression (Tomar and Asma, 2000). That study

showed that former smokers were more likely to have periodontitis than subjects who had never smoked, and current smokers were about four times as likely as subjects who had never smoked to have periodontitis. Among former smokers, the odds for periodontitis decreased with the number of years since giving up, while among current smokers, there was a dose-response relationship between cigarettes smoked *per* day and the odds for periodontitis.

1.9.1 Effect of smoking on the periodontal tissues

Several studies have shown that smoking can reduce the clinical signs of gingival inflammation in periodontitis and experimental gingivitis patients. This could be due to the potential of nicotine to cause vasoconstriction in the peripheral blood vessels (Clarke et al. 1981). Significantly lower GCF volume was found for smokers than non-smokers, in periodontitis patients (Kinane and Radvar, 1997). Holmes et al. (1990) compared the gingival crevicular flow between periodontally healthy smokers and non-smokers. Smokers tended to exhibit lower GCF volume than did non-smokers and lingual readings were not significantly different from buccal readings. These findings indicate that the effect of cigarette smoke on clinically healthy gingiva may be a result of vasoconstriction rather than a physical irritation.

Smoking was found to suppress overt clinical signs of gingival inflammation, as indicated by a reduced bleeding score in the smokers (Preber and Bergström, 1985). In addition, Darby et al. (2000) examined the periodontal status in chronic periodontitis and generalised aggressive periodontitis patients, and found that smokers in both disease groups exhibited significantly lower bleeding scores than non-smokers. A more recent study has confirmed earlier reports and has shown a suppressed bleeding on probing in smokers, by examining the hemorrhagic responsiveness in two populations of subjects, one of which were periodontitis patients and the other dental care attenders in general (Bergström and Boström, 2001). Bergström et al. (2000) showed that in individuals free of periodontal disease the association between smoking and gingival bleeding was weak. The authors speculated that the interference of smoking with the inflammatory responsiveness of the periodontium may not be attributed solely to the vasoconstriction effects of tobacco and its products, but that it may be a result of changes in the vascular dynamics and cellular metabolism induced by smoking (Bergström and Boström, 2001).

Danielsen et al. (1990) reported findings from a 21-day experimental gingivitis study conducted in periodontally healthy smokers and non-smokers. By day 21, both subgroups had equivalent amounts of plaque, but smokers developed fewer sites with gingivitis assessed clinically than non-smokers, possibly reflecting a reduced capacity of the host to induce an effective response to the plaque challenge. Lie et al. (1998b) assessed the bleeding tendency in smokers versus non-smokers and demonstrated that at the end of 14 days of experimental gingivitis smokers showed less bleeding on probing than non-smokers. Differences in bleeding indices between the two subgroups were seen for probing the marginal gingiva and to the bottom of the pocket as well. This suppressed inflammatory response of smokers to plaque accumulation implies that smokers should be studied as a separate group in experimental gingivitis trials. These findings indicate that smoking reduces gingival inflammation and thus masks early signs and symptoms of periodontal disease.

The harmful effects of cigarette smoking on periodontal health in subjects with generally good oral hygiene standards and dental awareness have been demonstrated (Bergström and Eliasson, 1987b). Both the number of pockets and probing pocket depth were significantly greater in smokers than in non-smokers and this finding existed even after taking into consideration age and oral hygiene. The same investigators demonstrated that the frequency of diseased sites and bone loss was significantly greater in current smokers than in non-smokers (Bergström et al. 2000). In addition, a dose-dependent realtionship was found between periodontal disease and smoking. The finding that former smokers exhibited less disease than current smokers, although worse than non-smokers, indicates that smoking cessation may be beneficial for the periodontium.

These results are in accord with those of Haffajee & Socransky (2001), who showed that current smokers had significantly more attachment loss, missing teeth, deeper pockets and fewer sites with bleeding on probing, yet similar levels of plaque and gingival redness than former smokers or subjects who had never smoked. In agreement with the findings of Bergström & Eliasson (1987a), this study revealed larger differences in pocket depths between smokers and non-smokers for the middle of the pocket depth range rather than the ends of the range. Age, pack-years and being a current smoker were strongly associated with mean attachment level. Significantly more bone loss was

observed at maxillary palatal sites and lower anterior teeth suggesting the likelihood of a local effect of cigarette smoking. Kamma et al. (1999) evaluated the impact of smoking on the clinical status of aggressive periodontitis patients. Smokers exhibited increased number of diseased sites and more severe periodontal destruction in terms of pocket depth, attachment loss and radiographic bone loss than did non-smokers, and this was observed mainly in maxillary anteriors and premolars. In a more recent study, cigarette smoking was associated with deeper periodontal pockets and the intra-oral distribution of the pockets suggested a local effect (van der Weijden et al. 2001).

Several studies have reported that plaque levels were similar between smokers and non-smokers (Kinane and Radvar, 1997; Kamma et al. 1999; Darby et al. 2000; Haffajee and Socransky, 2001), implying that the deleterious effects of cigarette smoking on the periodontal tissues are less likely to be related to compromised plaque control (Bergström and Eliasson, 1987a, 1987b).

Another study examined the serum cotinine levels in periodontitis patients and found a correlation between the cotinine levels and the severity of chronic periodontal disease (González et al. 1996). Variations in cotinine levels among "one pack a day" smokers were shown, suggesting that self-reporting of smoking status may not be reliable. This could result from the fact that some individuals may be untruthful when self-reporting, or that individual variations in smoking habits and variations among different cigarettes exist.

1.9.2 Subgingival microbial flora in smokers

After matching for age, sex, plaque and calculus in a sample of healthy middle-aged adults, one study found that the odds of having posterior proximal probing depth ≥ 3.5 mm were 5.3 times greater for smokers than non-smokers (Stoltenberg et al. 1993). The prevalence of five putative periodontal pathogens, as determined by immunofluorescence, did not differ between smokers and non-smokers and smoking was a stronger risk indicator for the presence of posterior proximal probing depth ≥ 3.5 mm than the detection of any of the five bacteria. The relationship between smoking and occurrence of periodontal pathogens was assesssed in 145 patients with severe periodontitis by a culture technique (Preber et al. 1992). The results of this study showed that there were no differences in the prevalence, counts and occurrence of

different combinations of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* between smokers and non-smokers.

In accordance with these findings, another study failed to show any differences in the prevalence of five putative periodontal pathogens between smokers and non-smokers (Darby et al. 2000). The organisms were detected in subgingival plaque using PCR in two different groups of patients, one with chronic periodontitis and the other with generalised aggressive periodontitis. Utilizing the checkerboard DNA-DNA hybridization technique, Boström et al. (2001) confirmed the previous data by showing that, in chronic periodontitis patients, smoking had little, if any, influence on the subgingival presence of several suspected periodontal pathogens. Lie et al. (1998a) demonstrated that the phenomenon that smokers presented with less bleeding than non-smokers after the induction of experimental gingivitis was not associated with microbiological differences between the two subgroups.

Conversely, Zambon et al. (1996) demonstrated by immunofluorescence that higher proportions of smokers harboured *B. forsythus*, *P. gingivalis* and *A. actinomycetemcomitans* than did non-smokers and they were infected with higher mean levels of the majority of the test bacteria than were non-smokers. Controlling for the severity of periodontal disease, former and current smokers were 1.5 times more likely to be infected with *B. forsythus* than non-smokers, and the greater risk seen in smokers for infection with *B. forsythus* was dose-related. Similarly, Kamma et al. (1999) compared the microbial profiles of smokers and non-smokers in a group of patients with aggressive periodontitis using a culture technique. The analysis of the subgingival plaques revealed that a variety of suspected periodontal pathogens, including *B. forsythus* and *P. gingivalis*, were found in significantly higher numbers and more frequently in non-smokers than smokers. In addition, another study demonstrated that former smokers exhibited a decreased risk of harbouring *A. actinomycetemcomitans* in saliva than non-smokers (odds ratio: 0.23), while current smokers displayed an increased risk of harbouring *T. denticola* in periodontal pockets than non-smokers (odds ratio: 4.61) (Umeda et al. 1998).

Differences in the composition of the subgingival microbial flora among various studies between smokers and non-smokers could be attributed to sampling techniques, detection

methods, characteristics of patient populations (such as sample size) and detection of different putative pathogens.

1.9.3 Immunological profiles of smokers

Smoking has been shown to have an adverse effect on fibroblast function (Raulin et al. 1988), chemotaxis and phagocytosis by neutrophils (Kenney et al. 1977; Kraal et al. 1977), and immunoglobulin production (Holt, 1987; Johnson et al. 1990). It has been shown that cigarette smoke results in reduced concentration of serum IgG antibodies (Andersen et al. 1982). The effects of smoking and tobacco on the immune system are summarised in a review, which highlights the deleterious effects of tobacco on the host response (Barbour et al. 1997).

Reduced serum antibody titres to *P. intermedia* and *F. nucleatum* were found in smokers (Haber, 1994). Smoking has been related to depresssed serum IgG_2 antibody in generalised aggressive periodontitis patients (Quinn et al. 1996). The significance of this finding is that antibody responses elicited by carbohydrate antigens, including LPS, have high quantities of IgG_2 (Scott et al. 1988). These antigens are commonly found on oral pathogens. In another study from the same laboratory, IgG_2 antibody levels were reduced in smoking chronic periodontitis patients and healthy controls of white race, but not in black subjects (Quinn et al. 1998). It was interesting to note that there was a relation between a smoking-related decrease in serum IgG_2 and an increase in periodontal destruction in white subjects. In accordance with previous findings, this study showed that smoking had an impact on serum immunoglobulin levels, but this effect was both race- and serum IgG subclass-specific.

Gunsolley et al. (1997) examined the combined effects of IgG allotype, race, smoking and periodontal diagnosis on IgG subclass levels. In black subjects smoking was only asssociated with lower concentrations of IgG_1 in chronic periodontitis patients and lower concentrations of IgG_2 in subjects with generalised aggressive periodontitis. However, in white subjects complex relationships existed between smoking and allotypic markers, but no influence of periodontal diagnosis was found. Thus, in addition to immunoglobulin allotype, smoking is associated with IgG subclass concentrations and, together with periodontal diagnosis and gender, all influence IgG subclass concentrations in black subjects. In conclusion, smoking can modify the

antibody response seen. Tangada et al. (1997) tested the hypothesis that smoking results in reduced IgG_2 antibody levels to *A. actinomycetemcomitans* in black generalised aggressive periodontitis patients but not in black localised aggressive periodontitis patients. The results confirmed the study hypothesis and also indicated that serum IgG_2 reactive with other antigens might not be reduced in this group of periodontitis patients. An interesting finding was that no significant differences in the anti-*A. actinomycetemcomitans* IgG_2 antibody levels existed between non-smoking generalised and localised periodontitis patients.

A more recent study examined the effect of smoking on the IgG titres and avidity to a panel of five putative periodontal pathogens in two subgroups of aggressive periodontitis patients, one group of untreated patients and the other of maintenance patients (Mooney et al. 2001). Median antibody titres to *A. actinomycetemcomitans*, *P. intermedia* and *T. denticola* were significantly lower in smoking maintenance patients, but not in untreated patients. Antibody avidity to *P. gingivalis* was also lower in the former patients but not in the latter. These results indicate that the maturation of the humoral immune response during treatment is compromised in smokers.

1.9.4 The effect of smoking on periodontal therapy

Boström et al. (1998) showed a predominance of smokers among patients exhibiting loss of bone height after five years of maintenance, and an increased level of TNF-α in the GCF of smokers. No differences in supragingival plaque and presence of periodontopathogens subgingivally were seen between smokers and non-smokers. Over the five years of monitoring, the proportion of smokers who dropped out of the study was higher, implying that there is lack of motivation in this group of patients. Kaldahl et al. (1996c) evaluated the effects of the level of cigarette consumption and a previous smoking history on the treatment outcome and on supportive periodontal treatment which lasted seven years. Heavy and light smokers demonstrated less pocket depth reduction, less gain in clinical attachment level and greater loss of horizontal attachment level than previous smokers and non-smokers following active and supportive treatment. No differences were seen in bleeding on probing among the groups and heavy smokers exhibited a higher percentage of sites with supragingival plaque than the other groups. These data suggest that past history of smoking does not have an adverse effect on the treatment outcome. The same investigators examined the yearly incidence

of sites with active periodontal disease losing clinical attachment level during a seven-year period of supportive treatment (Kaldahl et al. 1996b). They showed that patients with higher breakdown incidences tended to be smokers at the initial examination, suggesting that smoking cessation may be a therapeutic consideration in the long-term clinical treatment of periodontal patients.

Another study monitored the short-term effects of conventional periodontal treatment on the subgingival microbial flora in smokers and non-smokers (Preber et al. 1995). Smokers had significantly greater mean pocket depth as well as greater number of diseased sites at baseline. The treatment resulted in similar plaque and gingival indices for both subgroups, but in a significantly smaller pocket reduction in smokers than non-smokers. There were no microbiological differences between the two groups, and the authors speculated that the compromised clinical outcome seen in smokers may be due to factors related to the host response rather than to microbial infection. A further study showed that there were no differences in baseline clinical parameters between smokers and non-smokers (Renvert et al. 1998). However, six months after treatment bleeding scores were greater and pocket depth reduction was smaller in smokers than non-smokers. Although, *A. actinomycetemcomitans* was more difficult to eradicate among smokers than non-smokers, there were no significant differences in the microbiological responses between the two groups.

The effect of cigarette smoking on the outcome of scaling and root planing and adjunctive antimicrobial treatment has been evaluated (Kinane and Radvar, 1997). Non-smokers showed greater pocket depth reduction and a trend for greater gain in attachment levels, indicating that a greater degree of recession occurred in smokers rather than non-smokers. The percentage of sites with bleeding on probing after treatment was not significantly different between the two groups, and this was also the case with the plaque scores. No differences in the baseline plaque scores were seen between the two groups, suggesting that the compromised clinical outcome in smokers could be attributable to factors other than the level of plaque control. Haffajee et al. (1997a) reported that pre-treatment *P. gingivalis*, *B. forsythus* and *T. denticola* were equally prevalent among current and past smokers and subjects who had never smoked, and decreased significantly after periodontal therapy in past smokers and in subjects who had never smoked but increased in current smokers. Current smokers had deeper

pockets and more attachment loss than past smokers or subjects who had never smoked, both pre- and post-treatment. In summary, the findings presented in this section strongly relate smoking to a compromised clinical outcome and suggest that smoking cessation can have beneficial effects on periodontal health and management of periodontal diseases.

1.10 Polymerase chain reaction and detection of putative periodontal pathogens

PCR is a rapid and highly sensitive technique which has been used for the detection of bacterial DNA sequences. PCR is highly specific and relatively inexpensive. It is not as labour-intensive as other methods and it does not rely on viable cells. With these characteristics in mind, PCR can be more advantageous than other techniques such as culture methods, immunodiagnostic methods and chromosomal and cloned DNA probes (Ashimoto et al. 1996; Riggio et al. 1998). Studies from our laboratory showed that the sensitivity of PCR for the leukotoxin A and fimbrillin gene was 50 cells of *A. actinomycetemcomitans* and *P. gingivalis*, respectively (Riggio et al. 1996), and for the 16S rRNA gene was 100 cells of *P. intermedia* (Riggio et al. 1998).

Slots et al. (1995) showed that the proportions of subgingival plaque samples positive by PCR ranged from 52.8% (*A. actinomycetemcomitans*) to 91.4% (*B. forsythus*) in a group of patients with advanced periodontitis. Another study from the same laboratory found a prevalence of 30% for *A. actinomycetemcomitans*, 86% for *B. forsythus*, 70% for *P. gingivalis*, 58% for *P. intermedia*, and 54% for *T. denticola* in subginigval plaque of advanced periodontitis patients (Ashimoto et al. 1996). A further study detected *P. gingivalis* in 25% of the healthy subjects and in 79% of the periodontitis patients, emphasising the possible pathogenic role of this organism in periodontitis (Griffen et al. 1998). *A. actinomycetemcomitans* and *P. gingivalis* were detected in subgingival plaque samples of refractory periodontitis patients at frequencies of 44% and 58%, respectively (Wahlfors et al. 1995) and *B. forsythus* was detected in 89.7% of periodontitis patients (Meurman et al. 1997). In another study, 78.5% of periodontitis patients were found to be positive for the fimbrillin gene of *P. gingivalis* (Amano et al. 1999).

Investigations from our laboratory revealed that 40% and 28% of chronic periodontitis patients harboured *A. actinomycetemcomitans* and *P. gingivalis* respectively (Riggio et al. 1996), and 52% of the patients possessed *P. intermedia* (Riggio et al. 1998). More

recently, the prevalence of five putative periodontal pathogens was compared between patients with chronic periodontitis and generalised aggressive periodontitis by PCR (Darby et al. 2000). In that study, 54.4% of chronic periodontitis patients harboured *P. gingivalis*, 3% *A. actinomycetemcomitans*, 72.9% *P. intermedia*, 54.4% *T. denticola*, and 63.6% *B. forsythus*. The prevalence of these organisms in generalised aggressive periodontitis patients was as follows: 62.5% of patients were positive for *P. gingivalis*, 20.8% for *A. actinomycetemcomitans*, 79.2% for *P. intermedia*, 45.8% for *T. denticola*, and 91.7% for *B. forsythus*.

Umeda et al. (1998) showed that risk indicators for harbouring six suspected periodontal pathogens as detected by PCR included race / ethnicity, age, gender, time of residency in the United States, smoking, frequency of professional dental care and existing periodontal disease. The data reported in this study revealed that the prevalence of *A. actinomycetemcomitans* in the advanced periodontitis patients was 35.7% in Caucasians, 30.8% in African-Americans, 50.0% in Asian-Americans, 58.3% in Hispanics and 35.7% in all subjects. *P. gingivalis* was detected in 50.0%, 69.2%, 75.0% and 83.3% of the above mentioned ethnic groups respectively, and in 56.3% of all subjects. *P. intermedia* was detected in 71.4%, 38.5%, 87.5% and 91.7% of the four ethnic groups respectively, and in 46.2% of all subjects. *T. denticola* was found in 78.6%, 84.6%, 87.5% and 100% of the ethnic groups respectively, and in 71.9% of all subjects. *B. forsythus* was prevalent in 85.7%, 84.6%, 87.5% and 100% of the different ethnic groups respectively, and in 63.3% of the total number of subjects.

Thus, differences in the detection frequencies of organisms by PCR among various studies could be due to variations in the patient populations, but also to differences in the sampling methods and in the laboratory analysis performed (primers used, primer annealing temperatures, primer sensitivity, sample processing, etc) and to operator variability.

1.11 Objectives of the study

The study presented in this thesis examined the efficacy of two treatment strategies, full-mouth scaling and root planing performed within 12 hours (FM-SRP) versus quadrant scaling and root planing performed over four visits at two-weekly intervals (Q-SRP). The primary aim of this study was to determine whether these methods of treatment had

different effects at six weeks after the completion of scaling and root planing and six months after the initiation of therapy, on:

- Clinical parameters (full-mouth and site-specific clinical indices);
- Humoral immune response dynamics (systemic and local antibody titres and antibody avidity);
- Subgingival microflora (detection of five suspected periodontal pathogens in subgingival plaques);

In addition, the clinical, immunological and microbiological parameters for each treatment strategy were compared before and after therapy.

Other aims of this study were to:

- Determine the relationship between severity of periodontal disease and immunological and microbiological parameters;
- Determine the relationship between clinical improvement and changes in immunological and microbiological responses;
- Determine the impact of smoking on clinical and immunological parameters and on the detection frequency of five periodontal pathogens in subgingival plaque;
- Determine whether during the period of quadrant scaling and root planing at two-weekly intervals, treated sites in quadrant one deteriorated clinically and microbiologically in the presence of non-scaled sites in the other quadrants;
- Test the hypothesis that each session of quadrant scaling and root planing and oral hygiene instructions had a beneficial effect on the clinical indices in the remaining untreated quadrants;
- Test the hypothesis that in the short-term, Q-SRP over four visits elicited an immune response that reached peak levels more gradually and was sustained at high levels for a longer period of time than that elicited by one session of FM-SRP.

CHAPTER 2

MATERIALS AND METHODS

2. Materials and methods

2.1 Clinical methodology

2.1.1 Patient selection

This study was approved by the Ethics Committee at Glasgow Dental Hospital and School. All participants were fully informed about the nature of the treatment and singed a consent form prior to recruitment.

60 patients were recruited from new referrals to the Department of Periodontology at Glasgow Dental Hospital between February 1999 and September 2000. These were untreated moderate to advanced periodontitits patients with no history of systemic disease or antibiotic therapy in the last three months. Each patient, who had at least two sites *per* quadrant with a pocket depth of 5 mm or greater and radiographic evidence of bone loss, entered into the study. A history of cigarette smoking was recorded and confirmed using the COZART serum cotinine assay kit (Abingdon, U.K.).

2.1.2 Clinical study design

Patients were randomly allocated into two groups:

- **Q-SRP group.** Received quadrant scaling and root planing (Q-SRP) over four visits at two-weekly intervals,
- **FM-SRP group.** Received full-mouth scaling and root planing (FM-SRP) within 12 hours.

Patients were randomly allocated into one of the two treatment groups based on a predetermined randomisation list made by computer. The Q-SRP group consisted of 20 patients (13 males and 7 females) with a mean age of 42.3 years (range 31-70) and the FM-SRP group consisted of 22 patients (12 males and 10 females) with a mean age of 47.5 years (range 36-67) (Table 4). All participants were of Caucasian ethnic origin except for two subjects who were Asians (one subject in the Q-SRP group and one in the FM-SRP group). Seven patients in the Q-SRP group and ten in the FM-SRP group were smokers. Smokers in the Q-SRP group consumed on average 13.6 cigarettes a day (range 5-20) and in the FM-SRP group 16.2 cigarettes a day (range 10-30). The mean number of teeth was 27 and 26 for Q-SRP and FM-SRP groups, respectively.

Scaling and root planing was performed under local anaesthesia. In the Q-SRP group, root planing started with the upper right quadrant, to be continued clockwise over four

Table 4. Demographic details for Q-SRP and FM-SRP patients.

	No. of subjects	No. of teeth*	Age*	Males	Females	Smokers
Q-SRP	20 19 Caucasians 1 Asian	27 (23-31)	42.3 (31-70)	13	7	7
FM-SRP	22 21 Caucasians 1 Asian	26 (18-32)	47.5 (36-67)	12	10	10

* Mean (min-max)

Q-SRP = quadrant scaling and root planing

FM-SRP = full mouth scaling and root planing.

visits at two-weekly intervals. This order was kept consistent for all patients in the Q-SRP group, so that equal healing time was allowed for the treated quadrants. In the FM-SRP group, root planing was performed in one lower and one upper quadrant at the morning clinical session, to be continued in the other half of the dentition at the afternoon session, on the same day. Time spent for scaling each quadrant was approximately one hour. FM-SRP was performed over four hours in total, two hours at the morning session and two hours at the afternoon session on the same day. Patients in both groups were advised not to use antiseptic mouthwash during the course of treatment so that plaque control was achieved solely by optimal toothbrushing. No teeth were extracted during therapy in order to avoid bias.

The subjects were assessed with conventional full-mouth periodontal pocket charts at three time points: 1) baseline (BAS), 2) reassessment one (R1); six weeks after the last session of scaling and root planing, 3) reassessment two (R2); six months after the initiation of therapy. Furthermore, one site *per* quadrant with the deepest pocket depth (PD) (not less than 5 mm deep) was selected from each patient at baseline for collection of GCF, subgingival plaque samples and clinical data. From these selected sites, clinical data and samples were collected at baseline, at R1 and R2. In addition, for the Q-SRP group site-specific clinical indices were collected at each scaling session during the active phase of treatment.

The clinical design of the study was as follows:

Screening visit; the suitability for patient recruitment was confirmed and a signed consent form was obtained. Patients were randomly allocated into one of the two treatment groups.

Baseline; clinical indices and samples were collected.

Visit 1 (1 week later approximately); quadrant root planing for the Q-SRP group and full-mouth root planing for the FM-SRP group were performed under local anaesthesia.

Visit 2 (2 weeks later); sample collection for both groups, Q-SRP and oral hygiene instructions (OHI) were given to the Q-SRP group, whereas only OHI were given to the FM-SRP group.

Visit 3 (2 weeks later); same as visit 2.

Visit 4 (2 weeks later); sample collection for both groups, Q-SRP and OHI was given to the Q-SRP group whereas clinical reassessment was performed for the FM-SRP group.

Visit 5 (6 ± 1 weeks later); sample collection was carried out for both groups. Clinical reassessment was performed for the Q-SRP group. At this visit, maintenance scaling was performed in both groups.

Visit 6 (12 ± 2 weeks later); sample collection and clinical reassessment were carried out for both groups.

Subsequent to the one-day FM-SRP, patients in this treatment group were recalled at two-weekly intervals for OHI and blood collection. Effectively, patients in both treatment groups were seen at equal time points and received an equal amount of OHI and motivation. The timings of clinical interventions are summarised in Table 5.

2.1.3 Site selection

The following criteria for site selection were adopted: non-adjacent sites were selected so that there would be no interference between sites during plaque and GCF sampling. Mesial sites were preferred to distal sites since they were more accessible, with the deepest site being preferred. On the lower teeth, buccal and labial sites were preferred to lingual sites, since these sites were less prone to saliva contamination of the GCF sample. Single-rooted teeth were preferred over multi-rooted teeth. No sites with furcation or endodontic involvement were selected. Moreover, sites adjacent to teeth with poor prognosis, such as those with deep pockets proximal to the apex or with complications of endodontic or periodontic lesions were not included.

2.1.4 Sampling procedures

A blood sample was collected from the ante-cubital vein at each visit. Six blood samples in total were taken from patients in both groups.

Table 5. Timeline for clinical interventions.

Q-SRP GROUP

Baseline	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 (R1)	Visit 6 (R2)
A-E	F-G / I	A / E-G	A / E-G	A / E-G	A-F / J	A-F
0	1 week	3 weeks	5 weeks	7 weeks	13 ± 1weeks	25 ± 2 weeks

Q-SRP = quadrant scaling and root planing

FM-SRP GROUP

Baseline	Visit 1	Visit 2	Visit 3	Visit 4 (R1)	Visit 5	Visit 6 (R2)
A-E	H / F/ I	A / F	A / F	A-F	A / F / J	A-F
0	1 week	3 weeks	5 weeks	7 weeks	13 ± 1weeks	25 ± 2 weeks

FM-SRP = full mouth scaling and root planing

Key to table:

A: Blood sample (3 x 7ml tubes)

B: GCF samples

C: Plaque samples

D: Pocket chart (PD, AL, BOP)

E: Site-specific clinical assessment (PD, RAL, MGI, PI, BOP, Sup)

F: OHI

G: Q-SRP

H: FM- SRP

I: Questionnaire

J: Maintenance scaling

At baseline, R1 and R2, the following samples were collected from the study sites in the order listed:

1. Modified Gingival Index (MGI) and Plaque Index (PI).

2. GCF samples. GCF volume was determined using the Periotron 6000 (Oraflow Inc., Plainview U.S.A.).

3. First set of pocket depth (PD) measurements as determined by Florida probe (Florida Probe Corporation, Florida, USA), an electronic pressure sensitive probe.

4. Bleeding on probing (BOP) and suppuration (Sup).

5. The second set of PD measurements was recorded.

6. After changing the handpiece of the electronic probe, the first set of relative attachment level (RAL) measurements was taken.

7. The second set of RAL measurements was collected.

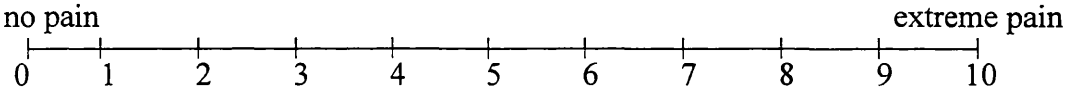
8. A subgingival plaque sample was collected from each site for PCR analysis.

9. Finally, a 20 ml venous blood sample was collected in order to obtain serum.

After the first session of scaling and root planing an anonymous questionnaire was given to participants of both groups to evaluate post-treatment complications (Figure 2). Day one was 24 hours after the first session of quadrant or full-mouth scaling and root planing, whereas day two was between 24 and 48 hours after the first session of scaling and root planing. The discomfort a patient experienced was rated on a 10 cm horizontal visual analogue scale with marks every centimetre (cm) (Figure 2). The cross mark placed by the patient was scored to the nearest cm, resulting in a score between zero (no pain) and ten (extreme pain). The percentage of patients taking analgesics, the number of painkillers and body temperature (thermometer placed at the axilla for five minutes) were also recorded. The occurrence of cold sores or oral ulcers was reported by the

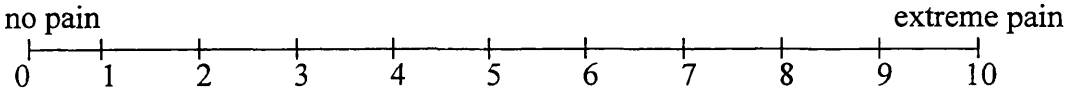
Figure 2. Assisted questionnaire.

- **Day 1, evening:**



Number of painkillers: .....
Body temperature: .....

- **Day 1, next morning:**



Number of painkillers: .....
Body temperature: .....

- **Day 2, evening:**

Number of painkillers: .....
Body temperature: .....

- **Day 2, next morning:**

Number of painkillers: .....
Body temperature: .....

patient or recorded by the examiner at the following visit, two weeks later. At that visit, the completed questionnaire was obtained.

2.1.5 Clinical measurements

Clinical measurements were collected by the same operator (DA) who had no access to recordings of previous visits. Full-mouth pocket charts were completed at baseline, R1 and R2. Pocket depth (PD) and attachment level (AL) were determined at six sites *per* tooth to the nearest millimetre (mm) using a PC 12 probe (Hu-Friedy Mfg Co, Chicago, IL, USA). Bleeding on probing (BOP) was also recorded dichotomously as present or absent after PD probing on each arch. PD was determined as the distance between the gingival margin and the base of the pocket and AL was measured as the distance between the cementoenamel junction (CEJ) and the base of the pocket.

At these visits, clinical data were also collected from the selected sites. The Florida Probe was used for PD and RAL recordings (Gibbs et al. 1988). Clinical measurements were recorded from the computer screen by an assistant. The operator was unable to view these recordings, so that an unbiased collection of measurements was ensured. All clinical indices were stored on a specially designed sheet. MGI, PI, BOP, Sup, PD and RAL were recorded at each site.

For the Q-SRP group, in addition to clinical assessment at baseline and reassessments one and two, clinical measurements were also collected from the selected sites at each scaling session at two-weekly intervals. At these visits, clinical recordings were collected from the sites in the remaining untreated quadrants only. For example, at baseline, clinical parameters were determined at sites in all quadrants; at visit two, in the second, third and fourth quadrant; at visit three, in the third and fourth quadrant; at visit four, in the fourth quadrant prior to scaling.

2.1.5.1 Clinical indices

2.1.5.1.1 The Modified Gingival Index (MGI)

The modified Gingival Index (MGI) (Lobene et al. 1986) was used to assess gingival inflammation and is described below:

0 Absence of inflammation.

1 Mild inflammation; slight change in colour, little change in texture of any portion but not the entire marginal or papillary gingival unit.

2 Mild inflammation; criteria as above but involving the entire marginal or papillary gingival unit.

3 Moderate inflammation; glazing, redness, oedema and/or hypertrophy of the marginal or papillary unit.

4 Severe inflammation; marked redness, oedema and/or hypertrophy of the marginal papillary gingival unit, spontaneous bleeding, congestion or ulceration.

MGI was preferred over the Gingival Index (Löe and Silness, 1963) because it permits non-invasive evaluation of the severity of gingival inflammation and thus facilitates GCF sampling. In addition, it provides greater sensitivity to detecting alterations in the signs of gingival inflammation, as the scoring system ranges from zero to four.

2.1.5.1.2 The Plaque Index (PI)

The plaque index (PI) (Silness and Löe, 1964) was used for recording plaque accumulation. The scoring system for this index is as follows:

0 No plaque in the gingival area.

1 A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may only be recognised by running a probe across the tooth surface.

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

3 Abundance of soft matter within the gingival crevice and/or on the gingival

margin and adjacent tooth surface.

2.1.5.1.3 Pocket Depth (PD) and Relative Attachment Level (RAL) measurements

PD and RAL were recorded by the Florida Probe to the nearest 0.2 mm. This is an electronic pressure sensitive probe set at 25 grams. The system consists of a pocket depth and an attachment level handpiece, a foot switch, computer interface and portable personal computer (Figure 3). The measurements of PD and RAL were made electronically, using the 'pocket depth' and 'attachment level' handpieces respectively (Figure 4), and transferred automatically to the computer when the foot switch was pressed. The assistant recorded clinical measurements on paper.

The probe tip has a diameter of 0.4 mm with no visible graduations along its length and it reciprocates through a sleeve. The edge of the sleeve is the reference from which measurements were recorded. PD was recorded when the probe sleeve was brought into contact with the gingival margin of the tooth (Figure 5). The Florida Probe, disc probe handpiece has a disc which, during attachment level measurement, touches the biting / occlusal surface of the tested tooth. When the tested tooth was not aligned with the rest of the teeth in the arch or was proximal to teeth with prominent cusps (canine) then the occlusal / biting plane of the adjacent tooth was preferred as a reference point for RAL measurement. Thus, attachment level measurements did not rely on the identification of CEJ, which is often complicated by its subgingival location or the presence of the restorations.

PD and RAL were carefully measured keeping the probe tip parallel to the long axis of the tooth. PD measurements were taken at the same points as RAL recordings and readings were taken twice to improve accuracy. If there was a discrepancy of more than 1.0 mm between the two readings, then one more additional recording was taken and the mean of these two measurements that had less than a 1.0 mm difference was used (Clark et al. 1993). After each probing the probe tip was wiped with isopropyl alcohol.

2.1.5.1.4 Bleeding on Probing (BOP)

Bleeding upon probing (BOP) was scored dichotomously using the Florida Probe. Scoring was performed within 30 seconds after the first probing as either one (1: BOP present) or zero (0: BOP absent).

Figure 3. Florida probe monitor, computer interface, and foot switch.

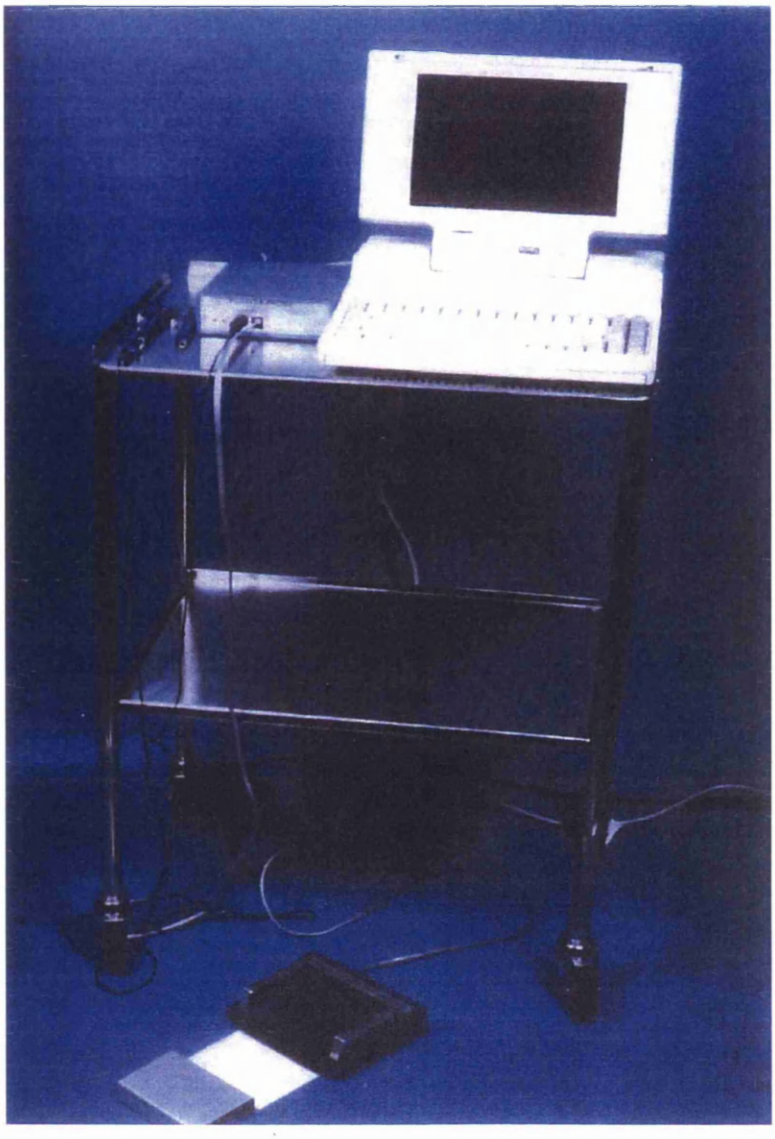


Figure 4. Florida probe attachment level and pocket depth handpieces.

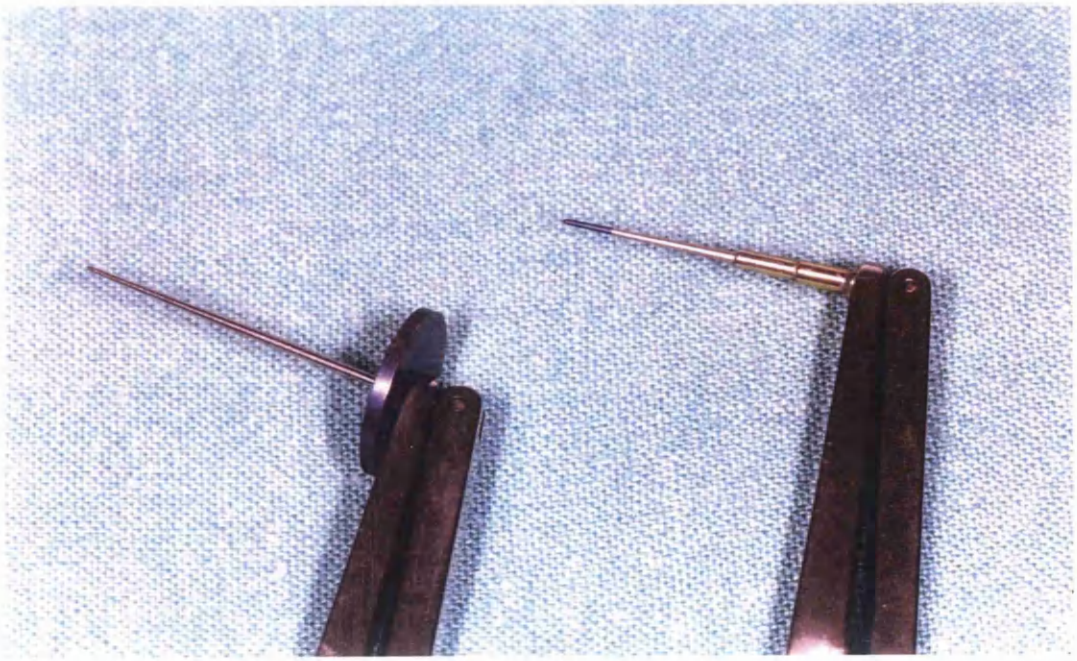
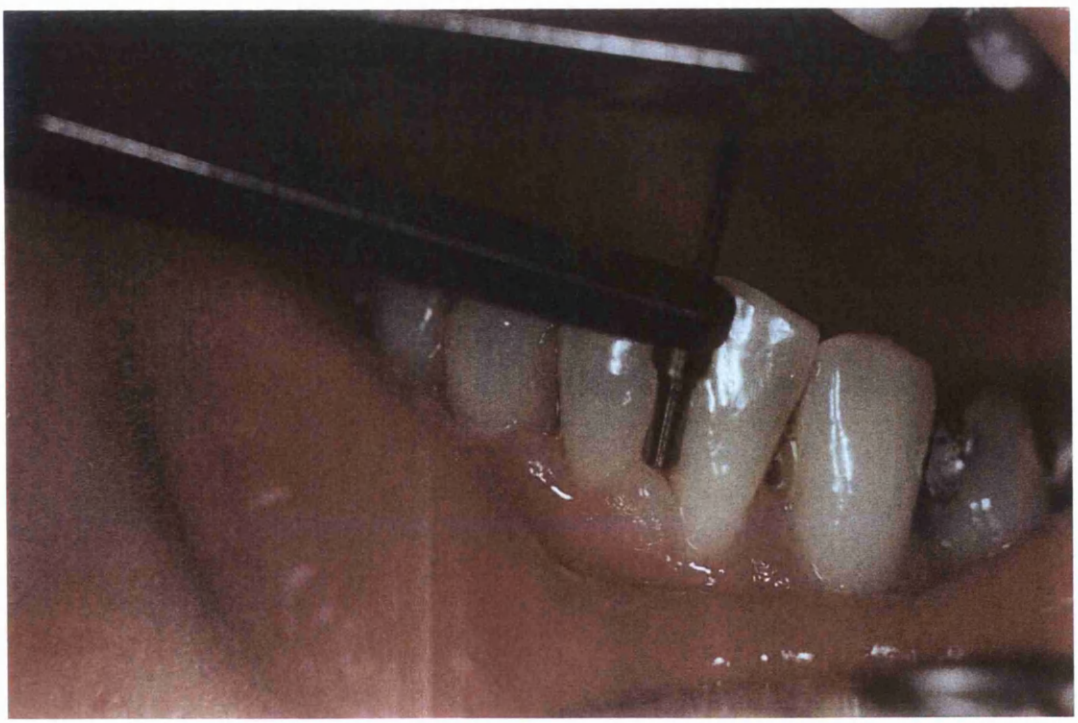


Figure 5. Florida pocket depth probe in use.



2.1.5.1.5 Suppuration (Sup)

Presence (score:1) or absence (score:0) of suppuration was recorded after applying gentle finger pressure on the gingival wall of the pocket at an apico-coronal direction.

2.1.6 GCF sampling

GCF samples were collected using Whatman grade 4 paper strips (2x13 mm) (Whatman Labsales Ltd. Maidstone, Kent). The paper strips were cut manually using a steel ruler and scalpel. Rubber gloves were worn during their preparation in order to avoid contamination of the strips by substances from the operator's hands. A line was drawn on each strip at 8 mm, indicating the length of the paper strip to be inserted between the Periotron jaws and that part of the strip to be used for GCF sampling. The strips were then placed in glass universal bottles for autoclaving and storage.

In the clinic, GCF was sampled after PI and MGI were determined but prior to any other clinical recordings which could cause irritation of the tissues and serum contamination of the sample. Such recordings included PD, RAL measurements, BOP and suppuration. The site being sampled was gently air-dried in an apico-coronal direction and supra-gingival plaque deposits were removed. The area was carefully isolated with cotton rolls and a saliva ejector, to prevent samples being contaminated by saliva. The paper strip was inserted into the crevice to the bottom of the pocket until mild resistance was felt, taking care in order to avoid mechanical injury of the tissues (Mann, 1963). By the intracrevicular method of GCF collection, the fluid volume is a reflection of the GCF volume and the pocket depth as well. Then, the strip was left in the crevice for 30 seconds and was discarded on visual signs of serum contamination (Lamster et al. 1985) (Figure 6). After GCF collection, the paper strip was transferred to the chairside-located Periotron 6000 for the quantification of fluid volume (Figure 7). The jaws of the Periotron were wiped with pure methanol between each reading. The strips were then stored on ice in patient- and site-labelled sterile tubes and stored at -70°C until required.

2.1.7 Blood sampling

Venous blood was collected from the ante-cubital fossa by venipuncture using the Vacutainer system (BD Vacutainer^{TM}, Plymouth UK). Three tubes of 7 ml of venous blood were collected.

Figure 6. Placement of filter paper strips in the gingival crevice for GCF collection.

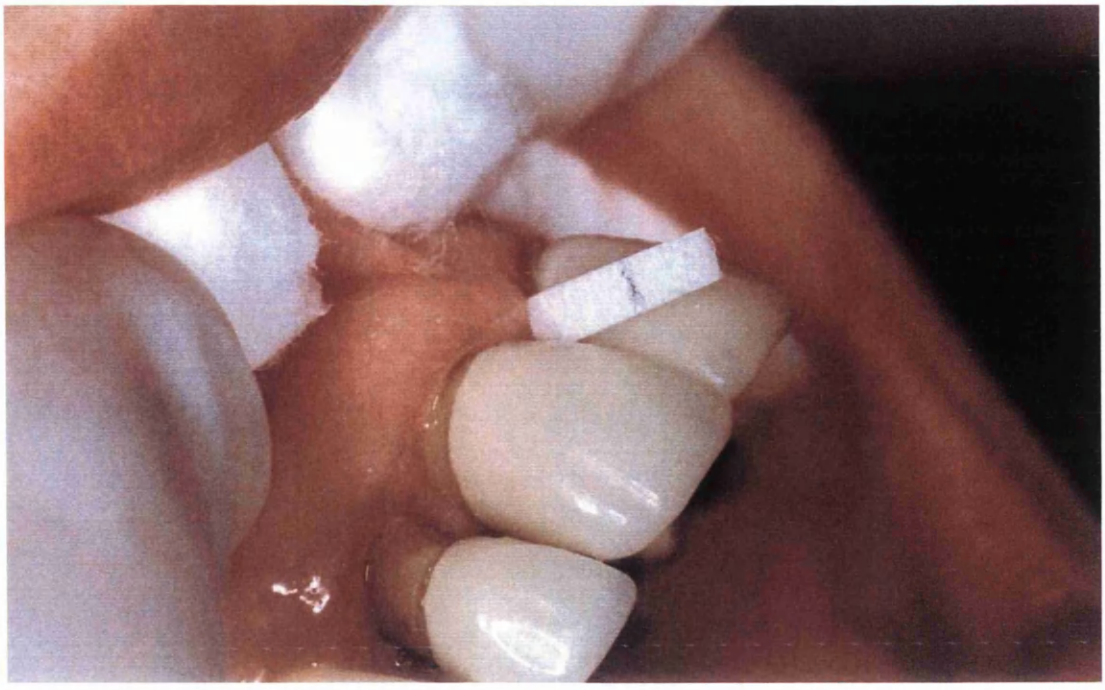


Figure 7. Periotron 6000.

2.1.8 Plaque sampling

After ensuring that no supragingival plaque deposits were present, the sites were isolated with cotton rolls and gently air-dried. Subgingival plaque samples were taken with a single vertical stroke, using a sterile hoe for each sample to prevent cross-contamination. Care was taken to access the most apical part of the pocket with the hoe. Samples were stored on ice in sterile micro-centrifuge tubes containing 0.5 ml sterile MilliQ grade H_2O (Millipore U.K. Limited, Watford, England).

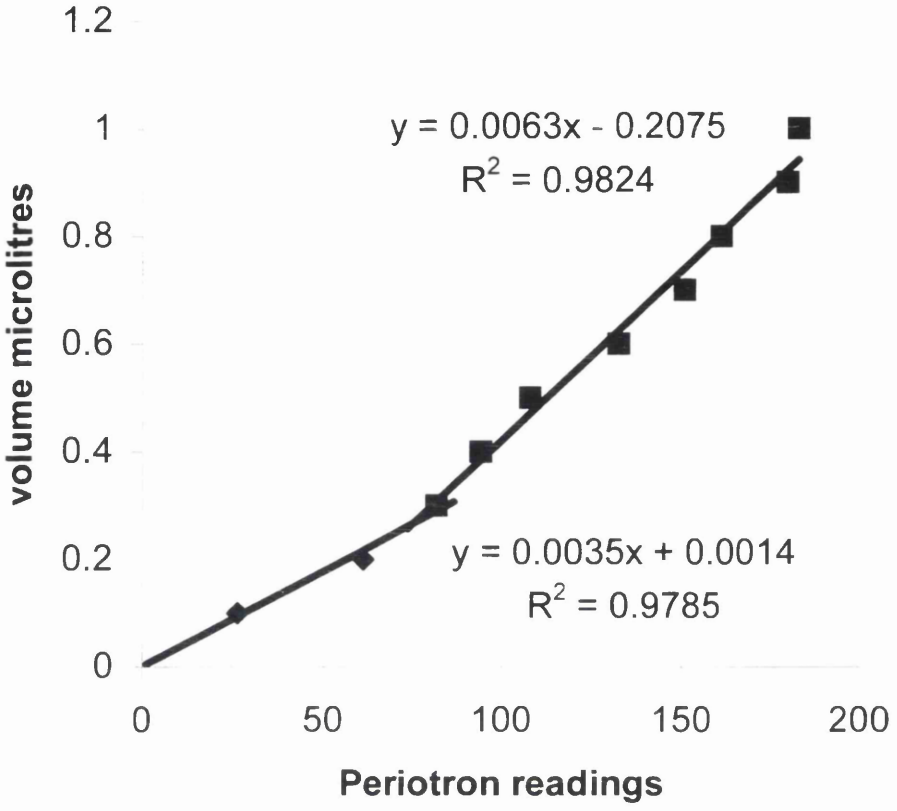
2.2 Experimental Methodology

2.2.1 Calibration of the Periotron 6000

In order to transform the Periotron digital readings for each paper strip into volumes and also assess the accuracy of the instrument, a calibration curve was constructed for the Periotron 6000. The Periotron was calibrated each time prior to GCF collection in order to minimise daily variability in the room's temperature and humidity. Before the Periotron was used the machine's digital display was adjusted to zero using a blank paper strip. GCF volumes were determined from the Periotron readings, using the equation $V = a + b \times p$, where V = GCF volume, a= intercept, b = slope and p = the Periotron reading. The curve was split into two parts, the upper and the lower as described by Lamster et al. (1985) and two lines were fitted by regression as shown in Figure 8. In the graph shown, the lower part included volumes of 0.1 to 0.3 µl while the upper part included volumes of 0.3 to 1.0 µl. GCF volumes were determined from the Periotron readings from the appropriate part of the Periotron calibration curve using the relevant derived formula.

Serum : phosphate-buffered saline (PBS) (1:1) was selected as the calibrating fluid since GCF with the majority of its constituents represents a dilution of serum (Adonogianaki, 1992). Known volumes of a serum : PBS (1:1) mixture were delivered to Whatman grade 4 paper strips with a Hamilton (0-1.0 µl) microsyringe (McQuiklin & Co, Glasgow, UK) in a range of volumes from 0.1 to 1.0 µl at 0.1 µl increments. Each measurement was performed three times and the mean value for each volume was used in a linear regression analysis from which the slope and intercept were used to determine the volumes of GCF collected. The digital display of the Periotron 6000 was zeroed before each measurement and the electrodes of the machine were carefully wiped with pure methanol and dried using Whatman grade 1 filter paper (Chapple et al. 1995). The GCF volumes were

Figure 8. Calibration curve for the Periotron 6000.



The determination of volumes of GCF from the Periotron 6000 readings. Linear regression analysis was used to fit lines to the data. The data from each analysis was resolved into two lines (0 to approx. 0.3 µl and 0.3 to 1.0 µl). The slope, intercept and correlation coefficient are shown (bottom right corner for the lower part and top left corner for the upper part of the curve).

determined from the calibration curve that was constructed based on Periotron readings taken on the same day as the GCF samples. Only two out of the 168 GCF samples that were collected displayed volume beyond the measuring range of the Periotron 6000 and they were arbitrarily assigned a volume of 1.6 µl.

2.2.2 GCF elution

GCF samples were eluted into 1ml of incubation buffer at room temperature using a rotary mixer for one hour. The strips were discarded and the eluates were aliquoted into eight tubes and stored at -70°C. The tubes were coded so that the laboratory analysis was performed in a blind manner.

2.2.3 Processing of sera samples

Each blood sample was allowed to clot overnight in tubes with no additive and then centrifuged at 2000 rpm for 10 minutes. The serum was aliquoted at a volume of 1 ml and stored into labelled microcentrifuge tubes at -70°C for further analysis. The aliquots were then coded so that the laboratory analysis was performed in a blind manner.

2.2.4 Processing of plaque samples

Plaque samples were vortex mixed for 30 seconds and stored in a coded sterile tube at -70°C until required. The PCR analysis was performed blindly. Once thawed the plaque sample was vortex mixed for 30 seconds and an aliquot was taken, for subsequent use in the PCR analysis. Lysates of plaque samples were prepared by boiling for 10 minutes.

2.2.5 Preparation of microorganisms and coating of plates

A. actinomycetemcomitans, *P. gingivalis*, *P. intermedia*, *B. forsythus* and *T. denticola* were prepared for coating the ELISA plates. *A. actinomycetemcomitans* strain Y4 was grown on blood agar plates and harvested after 24 hours. *P. gingivalis* strain W50, *P. intermedia* strain ATCC 25611, and *B. forsythus* strain ATCC 43037 were grown on fastidious anaerobe agar and harvested after seven days. The organisms were harvested with swabs and dispersed into PBS containing 0.1 mM disodium EDTA (PBSE). They were then washed once in PBSE and fixed overnight in 10% formal saline. *T. denticola* strain ATCC 35405 cells were grown, fixed and kindly donated by Dr C. Wyss, Zurich, Switzerland. After washing twice with PBSE, the fixed organisms were re-suspended in coating buffer (CB). The organisms were further washed once with coating buffer, and

were used to coat the plates at bacterial concentrations determined by the following optical densities (OD_{600}): 0.02 for *A. actinomycetemcomitans*, 0.05 for *P. gingivalis*, 0.05 for *P. intermedia*, 0.02 for *B. forsythus*, and 0.001 for *T. denticola*.

These optical densities were determined as optimal after coating the same plate with one bacterium at three different bacterial concentrations. In brief, an ELISA was carried out after adding control serum serially diluted from 1/100 to 1/25600 in IB. Then the EU readings were plotted against the serum dilutions at each coating density and linear regression analysis was performed. The coating giving the lowest background and the best correlation (R^2) was selected.

During the period of ELISA analysis, a new batch of *B. forsythus* of the same strain as previously used was utilised to coat the microtitre plates. In order to standardise the conditions of the assay, a plate was divided into two and each half was coated with the old and the new batches of the bacterium. Control serum serially diluted from 1/100 to 1/25600 in IB was then added and the reference line was calibrated with reference to the previous dilution factor.

2.2.6 ELISA buffers

The buffers employed in ELISA assays were as follows

1. Coating buffer (CB): 1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ was dissolved in 800 ml distilled H_2O. The pH was adjusted to 9.6 at just under 1L, by adding 0.1M HCl, and made up to 1L in a volumetric flask, with distilled H_2O. It was stored in sterilised bottles at 4°C for no longer than one week.

2. PBS: 8 g NaCl, 1.44 g $Na_2HPO_4.2H_2O$, 0.2 g KH_2PO_4, 0.2 g KCl, was dissolved in 800 ml of distilled H_2O, made up to 1L and stored at room temperature (pH 7.4).

3. Incubation buffer (IB): PBS with the addition of 0.5 g Tween 20 (SIGMA, Poole, Dorset) and 1g of lyophilised bovine serum albumin (BSA) (SIGMA, St. Louis, USA) (pH 7.4). BSA was layered on the surface until dissolved without mixing. It was stored at 4°C for maximum of one week.

4. 10 x Wash buffer (PBST): 10 times the concentration of IB (nil BSA) and stored at room temperature. It was diluted 1/10 immediately prior to use.

5. PBSE: PBS with the addition of 0.1 mM disodium EDTA (pH 7.4) and stored at room temperature.

2.2.7 Control serum for the ELISA

Standards for the range of ELISA analyses were obtained by initially testing sera from 33 patients with advanced periodontitis attending Glasgow Dental Hospital and School but not participating in the current study. ELISA analyses indicated which sera had high titres to the five putative periodontal pathogens. Then, these sera were serially diluted and assayed on the same plate by ELISA to test for linearity. The serum that gave the highest reading combined with the best linearity was selected as the control serum for that particular microorganism and was consistently used in all the experiments.

2.2.8 Enzyme-Linked Immunosorbent Assay (ELISA) of sera

Specific antibody titres were measured by enzyme-linked immunosorbent assay (ELISA) as previously described (Ebersole et al. 1980), using formalised whole cells at an absorbance that was previously determined as optimal to coat microtitre plates. Immulon 1 plates (Dynatech) were used because of their low protein-binding characteristics. The plates were manually pre-washed three times with coating buffer and coated with 100 µl *per* well of whole cells. Plates were stored at 4°C overnight. Controls for each aspect of the ELISA process were arranged in the wells around the outside of the plates.

After coating, the plates were washed five times with 250 µl of wash buffer using an automatic washer (Dynex Ultrawash Plus, Ashford, England). Then they were treated with 100 µl *per* well of incubation buffer (IB) containing 5% skimmed milk (Marvel, Premier Beverages, Stafford, UK) for 30 minutes at 37°C to remove background binding. The plates were washed again five times before the addition of sera. Serum serially diluted from 1/100 to 1/25600 in IB was used as a reference positive control serum. Serum derived from the study patients was added at a dilution of 1/200. 50 µl of sera was added in each well and was incubated at 37°C for 90 minutes. Following incubation with sera, the plates were washed again five times. Subsequently, 100 µl *per*

well of biotin-conjugated anti-human IgG (Sigma, St. Louis, MI, USA) at 1/2000 dilution in IB was added and plates were incubated at 37°C for 60 minutes. The plates were then washed five times. Thereafter, they were incubated at 37°C for 60 minutes or at 4°C overnight with 100 µl *per* well extravidin-peroxidase (Sigma) at 1/2000 dilution. After washing (five times) the reaction was visualised using 100 µl *per* well tetramethylbenzidine (TMB) (Kirkegaard & Perry Laboratories, Maryland, USA) and stopped after 5-10 minutes using 50 µl 0.1 M HCl.

Optical densities were read using a Dynex Technologies MRX II plate reader at 450 nm with a 630 nm reference wavelength. All sera samples collected from the same patient at different time points (six samples in total) were assayed in duplicate and on the same plate. Correction was made for non-specific binding and the averaged duplicate results were read from a reference line derived from serial dilutions of the reference positive control serum. Results were expressed as ELISA units (EU) (Gmür et al. 1986; Mooney et al. 1993). The ELISA was standardised such that 1/200 dilution of the reference control serum gave a value of 1000 EU (Mooney et al. 1993).

2.2.9 ELISA analysis of GCF samples

The same ELISA methodology that was used for analysis of the sera samples was used for analysis of the GCF samples. 50 µl of GCF aliquot was used *per* well instead of sera and it was not serially diluted. The samples were also analysed in duplicate and the averaged duplicate results were read from a reference line derived from serial dilutions of the reference positive control serum. All the GCF samples from the same patient were tested together on the same plate. The results were expressed as EU/30sec (Mooney and Kinane, 1997).

Since the GCF assay was standardised against a dilution of 1/200 control serum and the GCF sample was eluted in 1 ml of buffer (approximately 1/1000 dilution), one should multiply the results by 1000/200 i.e. by a factor of five.

2.2.10 Avidity analysis

The dissociation assay to determine antibody avidity was performed in a similar manner to the ELISA for the serum analysis described above. After incubation with sera, the wells were treated with increasing concentrations of ammonium thiocyanate (0, 0.2, 1.0,

2.0, 3.0 M). Patient serum was tested in duplicate at a dilution of 1/20, and the thiocyanate dilutions were compared to a buffer blank with 100% binding. The plates were incubated at 37°C for 60 minutes, washed five times automatically, and then the ELISA continued as before. The concentration of thiocyanate as a molarity (M) required to dissociate 50% of the bound antibody was calculated by linear regression. This concentration was termed the 50% inhibitory dose (ID_{50}) and provided a measure of the relative avidity (MacDonald et al. 1988; Pullen et al. 1986). All the samples from the same patient were tested together on the same plate.

2.2.11 Polymerase chain reaction (PCR)

Table 6 lists the PCR primers used in the current study. Species-specific primers that were previously tested for cross-reactivity with other closely related species targeted the 16S rRNA of the bacteria. The specificity of the amplified products and therefore of the primers was confirmed in previous studies by Ashimoto et al. (1996) for *P. gingivalis*, *A. actinomycetemcomitans*, *T. denticola* and *B. forsythus*, and by Riggo et al. (1998) for *P. intermedia*. All primers were obtained from MWG-Bistech (Milton Keynes, UK). Primers were supplied in a lyophilised form and were re-suspended in sterile, molecular biology grade water at 1 μg/ml (MilliQ grade H_2O).

PCR amplification reactions were carried out in a reaction mixture of 100 μl consisting of 10 μl sample lysate and 90 μl of reaction mixture containing 1xPCR buffer (10mM Tris-HCl (pH 9.0 at 25°C), 1.5mM $MgCl_2$, 50mM KCl, 0.1%Triton® X-100), 2 units of Taq DNA polymerase (Promega, UK), 0.2 mm dNTPs (dATP, dCTP, dGTP, dTTP) and 50 pmol of each primer. The primers were separated from the other components of the reaction mixture by a layer of wax (DyNAwax, Flowgen, Lichfield, UK). The wax layer prevented the PCR reaction from starting until the wax had melted upon commencement of PCR cycling ("hot start" PCR). PCR cycling was carried out in an OmniGene thermal cycler (Hybaid, Teddington, England, UK).

The yield of PCR product for *P. intermedia* was greater at 55°C compared to 60°C. Therefore, 55°C was selected as the annealing temperature for this organism in subsequent reactions. The cycling conditions for *P. intermedia* and *A. actinomycetemcomitans* comprised an initial denaturation step at 94°C for 5 minutes, 40 amplification cycles of denaturation at 94°C for 1 minute, annealing of primers at 55°C

Table 6. Sequences, expected product size, target and references for PCR primers.

Primer pairs (5'-3')	Base position Amplicon length (bp)	Target	Reference
P. gingivalis AGG CAG CTT GCC ATA CTG CG ACT GTT AGC AAC TAC CGA TGT	729-1,132 (404)	16S rRNA	Slots et al. (1995)
A. actinomycetemcomitans ATG CCA ACT TGA CGT TAA AT AAA CCC ATC TCT GAG TTC TTC TTC	478-1,034 (557)	16S rRNA	Ashimoto et al. (1996)
P. intermedia CCT AAT ACC CGA TGT TGT CCA CA AAG GAG TCA ACA TCT CTG TAT CC	1,028-1,006 (855)	16S rRNA	Riggio et al. (1998)
T. denticola TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	193-508 (316)	16S rRNA	Slots et al. (1995)
B. forsythus GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACC T	120-760 (641)	16S rRNA	Slots et al. (1995)

for 1 minute and primer extension at 72°C for 1.5 minutes, followed by a final extension step at 72°C for 10 minutes. The cycling conditions for *P. gingivalis*, T. *denticola* and *B. forsythus* were as follows: initial denaturation at 94°C for 5 minutes, 35 amplification cycles of denaturation at 94°C for 1 minute, annealing of primers at 60°C for 1 minute and primer extension at 72°C for 1.5 minutes, followed by a final extension step at 72°C for 10 minutes as previously described by Ashimoto et al. (1996). The reaction products were either stored at -20°C or analysed immediately.

Strict procedures were employed when carrying out PCR. Separate rooms were used for sample preparation, setting up of PCR reactions and analysis of PCR products. Filter tips were used at all stages of the experiment, except when adding the sample to the reaction mixture when a positive displacement pipette was used. Negative and positive controls were included in each batch of samples being analysed by PCR. The negative control was a 90 µl reaction mixture with the sample replaced by 10µl of sterile water. The positive control with the exception of *T. denticola*, contained 10 ng of genomic DNA from the relevant organism in 90 µl of reaction mixture, with sterile water added to bring the volume to 100 µl.

Since it was not possible to grow *T. denticola* at the microbiology laboratory at Glasgow Dental Hospital and School, a synthetic *T. denticola* positive control was constructed. A small fragment of the *T. denticola* 16S rRNA gene (79 bp) was amplified using the following nucleotide primers (from 5'-3'): TAA TAC CGA ATG TGC TCA TTT ACA TAA AGG TAA ATG AGG AAA GGA GCT (base position from 193 to 244) and T CAA AGA AGC ATT CCC TCT TCT TCT TA (base position from 508 to 482).

2.2.12 Analysis of PCR products

10 µl of each reaction product was added to 1.5 µl of gel loading dye (0.25% bromophenol blue, 50% glycerol, 100mM EDTA pH 8.0), electrophoresed on a 2% agarose gel containing ethidium bromide (0.5 µg/ml) and visualised and photographed using an ImageMaster video documentation system (Pharmacia Biotech, St. Albans, UK). A 100 bp DNA ladder (Pharmacia Biotech) was used as a molecular weight marker.

2.3 Statistical analyses

Minitab (release 12.1, Minitab Inc., State College, PA) and SPSS (version 9.0, SPSS Inc., Chicago, IL, USA) statistical packages were used for the analyses of the data. After treatment positive values denoted a positive change whereas negative values a negative change. The level for declaring statistical significance was set at the 95% confidence level ($p<0.05$ for hypothesis testing).

2.3.1 Clinical data

For the full-mouth clinical indices (PD, AL and BOP), a mean score was derived for patient at each visit. BOP was expressed as the percentage of sites which bled on probing. The number of sites with PD $\geq$ 5 mm (No. of sites $\geq$ 5 mm), *per* patient and *per* visit was also determined. In the assessment of clinical indices at the four specific sites (PD, RAL) a mean score *per* site and *per* visit was given.

For the comparison between the Q-SRP and FM-SRP groups over the three visits, an ANOVA analysis with the General Linear Model (GLM) was applied for the full-mouth and the site-specific clinical indices. The average of the clinical indices (PD and RAL) collected from the four selected sites *per* patient was used for this analysis. Treatment modality, smoking status and visit number and their interactions were modelled as fixed factors and the patient as a random factor. The PD, AL, BOP and No. of sites $\geq$ 5 mm for all the sites or the PD and RAL for the selected sites were used as the response variables for each analysis. The initial model included the three main effects of treatment, smoking and visit, together with the three-way interaction of these factors. If the three-way interaction was not significant, this model would be rejected and the next model would consist of the three main effects and the two–way interactions. Similarly, if the two-way interactions were not significant, they would be rejected and the model consisting of only main effects would be examined. Therefore, the final model included only the main effects and any interactions that gave a significant finding.

The full-mouth clinical indices (PD, AL, BOP and No. of sites $\geq$ 5mm) were compared between the two treatments at baseline, R1 and R2, using the two sample t-test. For the site-based analysis, the two sample t-test was used to compare the site-specific PD and RAL between the two treatment groups at baseline, R1 and R2. Differences in BOP and Sup between the groups were analysed using the Chi squared test, except when expected

counts were less than five where Fisher's exact test was used. The Mann-Whitney test was applied to analyse MGI and PI differences between Q-SRP and FM-SRP groups. The changes of the site-specific clinical indices (PD, RAL, BOP, Sup, MGI, PI) at deep pockets (mean PD $\geq$ 7mm) and moderate to deep pockets (mean PD $<$ 7 mm) were compared between the two treatments at R1 and R2 from baseline, using the equivalent tests mentioned above.

For the analyses of the full-mouth clinical indices (PD, AL and BOP) before and after treatment within each group, the paired t-test was used. The No. of sites $\geq$ 5 mm was also analysed in the same manner. The site-specific clinical indices PD and RAL were analysed using the paired t-test. The McNemar test was used to analyse BOP and Sup and the Wilcoxon signed rank test was used for the analyses of MGI and PI before and after treatment. Although, non-parametric statistical analysis was performed for MGI and PI, the mean values of these parameters are shown for illustration of effects.

In order to determine whether any clinical deterioration occurred at sites in quadrant one (1Q) for the Q-SRP group over a period of six months, the site-specific clinical indices (PD, RAL, BOP) of 1Q were compared with those of quadrant four (4Q) at baseline and at R1 and R2. The two sample t-test was used for the comparison of PD and RAL and the differences in BOP between the two quadrants were analysed using the Chi squared test. Changes in PD and RAL with treatment (BAS-R1 and BAS-R2) for 1Q and 4Q were determined using the paired t-test. The changes in BOP within each quadrant were analysed using the McNemar test.

For the Q-SRP group, during the active phase of treatment (baseline to each scaling session) the changes in site-specific clinical indices of the untreated sites were determined using the paired t-test for the analyses of PD and RAL, the Wilcoxon signed rank test for MGI and PI and the McNemar test for BOP and Sup.

For the analysis of the completed questionnaires, the Mann-Whitney test and the two sample t-test test were used to compare the pain rating and the body temperature between Q-SRP and FM-SRP groups, respectively.

2.3.2 Immunological data

The immunological data were not normally distributed even after transformations were performed and therefore parametric statistical tests were not appropriate. The Mann-Whitney test was used for the comparison of serum and GCF IgG titres, and IgG avidity between the two groups at baseline and after treatment. This test analysed the data for detection of differences between the two groups at each visit (BAS, R1, R2) and for comparison of changes with treatment (BAS-R1, BAS-R2). Within each group, the Wilcoxon signed rank test was used to assess changes before and after treatment. The GCF volume data were found to have a skewed distribution throughout all clinical assessments. Therefore, GCF volume was analysed in a similar manner as IgG titres and avidity. Although, non-parametric statistical analysis was performed for GCF antibody titres and GCF volume, the mean values of these parameters are shown for illustration of effects. Spearman's rank correlation coefficient was used to assess the relationship between GCF volume and GCF titres before and after treatment.

For the determination and comparison of the humoral immune response dynamics between Q-SRP group and FM-SRP group, the Mann-Whitney test analysed and compared the changes in the IgG antibody titres and avidities at each visit from baseline (baseline against five additional visits). The Wilcoxon signed rank test was used to determine the immune response dynamics in each group by comparing baseline with each follow up visit.

Spearman's rank correlation coefficient was used to assess the relationship between immunological parameters (serum IgG titres and avidity) and full-mouth clinical indices (mean PD and mean No. of sites $\geq$ 5 mm). In addition, the subjects of this study were categorised as moderately and advanced diseased periodontitis patients based on whether they had mean PD and mean No. of sites $\geq$ 5 mm equal to or less than the median and greater than the median baseline values, respectively. Then serum IgG titres and antibody avidity were compared between these two subgroups of patients using the Mann-Whitney test. In a similar way, the changes in antibody titres and avidity were compared between patients who yielded moderate and greater clinical improvement using the Mann-Whitney test. Patients with mean changes in clinical indices between baseline and R2 equal to or less than the median changes were designated as showing moderate improvement while those with mean changes greater than the median values were designated as showing large

clinical improvement. For the site-specific analysis GCF antibody titres and GCF volume were compared between sites of deep pocket depth (mean PD $\geq$ 7mm) and moderate pocket depth (mean PD < 7mm) using the Mann-Whitney test.

2.3.3 Microbiological data

The presence or absence of differences in the prevalence of the microorganisms between Q-SRP and FM-SRP groups were analysed using the Chi squared test, except when expected counts were less than five where Fisher's exact test was used. The data were analysed for differences at each visit (BAS, R1, R2) and for comparison of changes with treatment (BAS-R1, BAS-R2). For each group, the McNemar test was used to compare the prevalence of specific organisms before and after treatment. For the patient-based analysis the patient was scored positive for one organism if at least one out of the four sites harboured this organism.

For the comparison of changes in the dichotomous variables (BOP, Sup and the presence or absence of a periodontal pathogen) between the two treatment groups, the Chi squared test compared reduction in the detection of a variable (coded as 1) against no change and / or increase in the detection of this variable (coded as 0). Sites that showed an increase in the presence of BOP, Sup or in the detection of a pathogen after treatment were very few and were combined with the sites that showed no change post-treatment to form a no improvement category. Although the results are shown as percentages, the raw data were used in the statistical analysis.

The Chi squared test was used to compare the microbiological findings of sites in quadrant one (1Q) with sites in quadrant four (4Q) before and after Q-SRP and the McNemar test was used to compare the prevalence of the organisms at R1 and R2 from baseline for 1Q and 4Q.

The prevalence of the bacteria at baseline was compared between moderately and advanced diseased periodontitis patients and between deep and moderate to deep sites as described earlier, using the Chi squared test, with Fisher's exact test correction. These tests were also used to compare the changes in the prevalence of the organisms at R2 from baseline between patients with moderate clinical improvement and patients with large clinical improvement.

The patients were divided into two groups according to whether they were positive or negative for an organism. Then the full-mouth clinical indices and serum IgG titres and avidities were compared between these two groups. For the site-specific analysis the sites were divided into two groups based on the presence or absence of a microorganism and the site-specific clinical indices and GCF IgG titres were compared between these two categories of sites. The clinical and microbiological analyses between the two subgroups of sites or subjects that were positive or negative for an organism were performed in a similar manner as previously described.

2.3.4 Effect of smoking on clinical, immunological and microbiological parameters

In general, the differences between smokers and non-smokers for Q-SRP and FM-SRP, were analysed for clinical, immunological and microbiological parameters after dividing the patients into two groups according to their smoking status. These parameters were analysed in a similar manner as described above. Smokers were compared against non-smokers in all subjects at baseline and in the Q-SRP and FM-SRP groups before and after treatment. Finally, smokers in the Q-SRP group were compared against smokers in the FM-SRP group to determine if there were differences in the various parameters between the two treatments among smokers.

CHAPTER 3

EFFECT OF SCALING AND ROOT PLANING ON CLINICAL PARAMETERS

3. Effect of scaling and root planing on clinical parameters

3.1 Results

3.1.1 Demographic details

Initially, 60 patients were recruited. Ten patients were excluded during the course of the study because they failed to attend their appointments on two occasions. Seven of them belonged to the Q-SRP group and the remainder to the FM-SRP group. Of the ten poor attenders, one had a car accident, one emigrated, two subjects failed to attend due to work commitments and the remaining six dropped out of the study for unknown reasons.

Therefore, 50 participants completed the study but a further eight were excluded at the end of the study because they took antibiotics during treatment. Three of them belonged to the Q-SRP group and five to the FM-SRP group. One of the participants was prescribed antibiotics for a tooth abscess and the others for reasons not related to periodontal treatment. Out of the 42 patients who completed the study, three participants in total were excluded from R2, but not from the study. The three participants were FM-SRP patients, two of whom could not attend R2 due to work commitments and one due to antibiotic therapy. In conclusion, the analyses of this study were based on 42 subjects ($N_{Q\text{-}SRP}$=20 and $N_{FM\text{-}SRP}$=22) and 168 sites ($N_{Q\text{-}SRP}$=80 and $N_{FM\text{-}SRP}$=88) at R1, but at R2 the analysis for the FM-SRP group was limited to 19 subjects and 76 sites and this should be noted when referring to the tables. No subjects were excluded from this investigation due to poor response to therapy.

3.1.2 Clinical findings before and after Q-SRP

Full-mouth and site-specific clinical indices were significantly reduced at R1 and R2 with a further clinical improvement between R1 and R2. Whole-mouth clinical indices showed a significant PD reduction of 1.7 mm and 1.8 mm at R1 and R2 from baseline, respectively ($p<0.001$) (Table 7). There was a gain in attachment of 1.1 mm at both R1 and R2 ($p<0.001$). A reduction in BOP of 54% was found at R1 and of 58% at R2 ($p<0.001$). The number of sites with PD $\geq$ 5mm was significantly reduced by 56% at R1 and by 60% at R2 ($p<0.001$). Further clinical improvement between R1 and R2 was seen for PD ($p<0.05$), No. of sites $\geq$ 5mm ($p<0.01$) and BOP ($p=0.057$).

Table 7. Changes in the whole-mouth clinical indices for Q-SRP at R1 and R2.

N=20	Baseline	R1	R2	Change (BAS-R1)	p– value (BAS-R1)	Change (BAS-R2)	p– value (BAS-R2)	p– value (R1-R2)
PD (mm)	4.4 ± 0.7	2.7 ± 0.4	2.6 ± 0.3	1.7 ± 0.6	<0.001	1.8 ± 0.7	<0.001	0.015
AL (mm)	5.0 ± 0.9	3.9 ± 0.9	3.9 ± 0.9	1.1 ± 0.5	<0.001	1.1 ± 0.6	<0.001	0.444
BOP (%)	71.0 ± 19.0	17.0 ± 9.0	13.0 ± 7.0	54.0 ± 18.0	<0.001	58.0 ± 19.0	<0.001	0.057
No. sites≥5 mm	69.0 ± 20.0	13.0 ± 12.0	9.0 ± 9.0	56.0 ± 20.0	<0.001	60.0 ± 20.0	<0.001	0.007

Mean ± sd

Q-SRP = quadrant scaling and root planing

PD = pocket depth; AL = attachment level; BOP = bleeding on probing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 8 shows the changes in the site-specific clinical indices post treatment. PD was reduced by 2.7 mm at R1 and by 2.9 mm at R2 ($p<0.001$). RAL improved by 0.8 mm at R1 and by 1.0 mm at R2 ($p<0.001$). The improvements between R1 and R2 were significant for both PD and RAL ($p<0.05$).

Of the 80 sites in total, BOP was present at 66 sites (82.5%) at baseline and decreased to 14 (17.5%) at R1 ($p<0.001$) and 11 (13.8%) at R2 ($p<0.001$). One site that was initially negative for BOP at baseline became positive at R1 and four sites at R2. Suppuration was present at 49 sites (61.3%) at baseline, at three sites (3.8%) at R1 ($p<0.001$) and at one site (1.3%) at R2 ($p<0.001$). Nevertheless one site formerly negative for Sup, suppurated between BAS and R1 and one site between BAS and R2. Significant reductions in MGI and PI were seen at R1 and R2 ($p<0.001$). There was also a significant improvement in MGI between R1 and R2 ($p<0.001$).

3.1.3 Clinical findings before and after FM-SRP

There was a continuous improvement for all clinical parameters at R1 and R2. The analyses of the whole-mouth clinical indices showed significant PD reduction of 1.6 mm, and 1.7 mm at R1 and R2, respectively ($p<0.001$) (Table 9). Attachment levels improved after treatment, with a gain of 1.1 mm at R1 and R2 ($p<0.001$). There was a 52% reduction in BOP at R1 and 58% at R2 ($p<0.001$). The mean number of sites with PD $\geq$ 5 mm was reduced by 53% at R1 and by 54% at R2 ($p<0.001$). Between R1 and R2 a statistically significant improvement in PD ($p=0.001$), BOP ($p<0.005$) and number of sites $\geq$ 5 mm ($p<0.005$) was also observed.

Clinical data collected from the selected sites showed that mean PD was reduced by 2.1 mm at R1 and by 2.6 mm at R2 ($p<0.001$) (Table 10). RAL improved by 0.7 mm at R1 and by 1.1 mm at R2 ($p<0.001$). Additional improvements in PD and RAL between R1 and R2 were small but statistically significant ($p<0.001$).

The site-specific clinical analyses showed that BOP was present in 69 sites (78.5%) at baseline and decreased to 18 (20.5%) at R1 ($p<0.001$) and nine (11.8%) at R2 ($p<0.001$). Between BAS and R1, five sites initially negative for BOP bled on probing and between BAS and R2, one site became positive for BOP. Suppuration was present in 57 sites (64.8%) at baseline and was reduced to three (3.4%) at R1 ($p<0.001$) and

Table 8. Changes in the site-specific clinical indices for Q-SRP at R1 and R2.

N=80	Baseline	R1	R2	Change (BAS-R1)	p– value (BAS-R1)	Change (BAS-R2)	p– value (BAS-R2)	p– value (R1-R2)
PD (mm)	6.2 ± 1.2	3.5 ± 1.1	3.3 ± 0.8	2.7 ± 1.3	<0.001	2.9 ± 1.3	<0.001	0.013
RAL (mm)	14.0 ± 2.3	13.2 ± 2.3	13.0 ± 2.2	0.8 ± 1.0	<0.001	1.0 ± 0.9	<0.001	0.005
BOP*	82.5	17.5	13.8	65.0	<0.001	69.0	<0.001	0.660
Sup*	61.3	3.8	1.3	57.5	<0.001	60.0	<0.001	0.500
MGI	2.5 ± 0.8	0.5 ± 0.5	0.2 ± 0.4	2.1 ± 1.0	<0.001	2.4 ± 0.9	<0.001	<0.001
PI	1.9 ± 0.7	0.9 ± 0.8	0.7 ± 0.7	1.1 ± 0.8	<0.001	1.2 ± 0.9	<0.001	0.130

Mean ± sd; *% of positive sites

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

PD = pocket depth; RAL = relative attachment level; BOP = bleeding on probing; Sup = suppuration; MGI = modified gingival index; PI = plaque index.

Table 9. Changes in the whole-mouth clinical indices for FM-SRP at R1 and R2.

N=22	Baseline	R1	R2	Change (BAS-R1)	p– value (BAS-R1)	Change (BAS-R2)	p– value (BAS-R2)	p– value (R1-R2)
PD (mm)	4.4 ± 0.6	2.8 ± 0.3	2.6 ± 0.2	1.6 ± 0.5	<0.001	1.7 ± 0.6	<0.001	0.001
AL (mm)	5.1 ± 1.0	4.0 ± 1.0	3.9 ± 1.0	1.1 ± 0.4	<0.001	1.1 ± 0.5	<0.001	0.100
BOP (%)	69.0 ± 17.0	17.0 ± 10.0	11.0 ± 6.0	52.0 ± 15.0	<0.001	58.0 ± 18.0	<0.001	0.002
No. sites≥5 mm	66.0 ± 27.0	13.0 ± 6.0	8.0 ± 5.0	53.0 ± 23.0	<0.001	54.0 ± 24.0	<0.001	0.003

Mean ± sd

FM-SRP = full mouth scaling and root planing

PD = pocket depth; AL = attachment level; BOP = bleeding on probing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 10. Changes in the site-specific clinical indices for FM-SRP at R1 and R2.

N=88	Baseline	R1	R2	Change (BAS-R1)	p– value (BAS-R1)	Change (BAS-R2)	p– value (BAS-R2)	p– value (R1-R2)
PD (mm)	5.9 ± 1.0	3.8 ± 0.9	3.3 ± 0.8	2.1 ± 1.1	<0.001	2.6 ± 1.3	<0.001	<0.001
RAL (mm)	13.9 ± 1.8	13.2 ± 1.7	12.7 ± 1.8	0.7 ± 0.9	<0.001	1.1 ± 1.0	<0.001	<0.001
BOP*	78.5	20.5	11.8	58.0	<0.001	68.0	<0.001	0.170
Sup*	64.8	3.4	4.0	61.4	<0.001	59.0	<0.001	1.000
MGI	2.5 ± 0.6	0.5 ± 0.5	0.3 ± 0.5	2.0 ± 0.8	<0.001	2.2 ± 0.8	<0.001	0.015
PI	2.0 ± 0.7	0.7 ± 0.6	0.6 ± 0.7	1.3 ± 0.9	<0.001	1.4 ± 0.9	<0.001	0.032

Mean ± sd; *%of positive sites

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

PD = pocket depth; RAL = relative attachment level; BOP = bleeding on probing; Sup = suppuration; MGI = modified gingival index; PI = plaque index.

three (4.0%) at R2 ($p<0.001$). One site initially negative for suppuration became positive between BAS and R2. MGI and PI significantly improved at R1 and at R2 ($p<0.001$). A significant further reduction in MGI and PI was noted between R1 and R2 ($p<0.05$).

3.1.4 Comparison of clinical indices between Q-SRP and FM-SRP groups

No statistically significant differences in any clinical index were seen between the two treatments at baseline. The GLM analysis revealed that there was no significant three-way or two-way interaction among the following fixed factors: the treatment strategies (Q-SRP and FM-SRP), smoking and visits (BAS, R1, R2), on the full-mouth clinical parameters ($p>0.05$) (data not shown). Similarly, no significant effect of treatment or smoking on the full-mouth indices was seen ($p>0.05$). This series of analyses showed that there was a statistically significant visit effect on PD reduction, gain in AL, BOP improvement and reduction of No. of sites $\geq$ 5 mm ($p<0.001$). There was also a significant (random) patient effect on the full-mouth clinical indices ($p<0.01$). The GLM analysis for the selected sites showed that there was a significant three-way interaction among the following fixed factors: the treatment strategy, smoking and visits on PD and RAL of the selected sites ($p<0.001$) (data not shown).

Table 11 depicts marked clinical improvement in the whole-mouth clinical indices after treatment with no differences in any clinical index between the treatment groups at any time point. The differences in the site-specific clinical indices between the two treatment strategies over time are shown in Table 12. A significantly greater PD reduction of 0.6 mm was seen for the Q-SRP group between BAS and R1 when compared to the FM-SRP group ($p<0.005$). However, no statistically significant differences were noted between the two treatment groups between BAS and R2. PD was reduced by 2.9 mm for the Q-SRP group and by 2.6 mm for the FM-SRP group at R2. When the differences between R1 and R2 were examined, there was an additional 0.3 mm PD reduction for the FM-SRP group ($p<0.05$). No significant differences in gain of RAL were found between the two groups at R1 and R2 from baseline. RAL gain was 0.8 mm and 0.7 mm between BAS and R1, and 1.0 mm and 1.1 mm between BAS and R2 for the Q-SRP and FM-SRP groups, respectively.

Table 11. Comparison of the whole-mouth clinical indices before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=20 $N_{FM\text{-}SRP}$=22		Baseline	R1	R2	Change (BAS-R1)	p– value (BAS-R1)	Change (BAS-R2)	p– value (BAS-R2)	p– value (R1-R2)
PD (mm)	Q-SRP	4.4 ± 0.7	2.7 ± 0.4	2.6 ± 0.3	1.7 ± 0.6	0.58	1.8 ± 0.7	0.5	0.4
	FM-SRP	4.4 ± 0.6	2.8 ± 0.3	2.6 ± 0.2	1.6 ± 0.5		1.7 ± 0.6		
AL (mm)	Q-SRP	5.0 ± 0.9	3.9 ± 0.9	3.9 ± 0.9	1.1 ± 0.5	0.99	1.1 ± 0.6	1.0	0.4
	FM-SRP	5.1 ± 1.0	4.0 ± 1.0	3.9 ± 1.0	1.1 ± 0.4		1.1 ± 0.5		
BOP (%)	Q-SRP	71.0 ± 19.0	17.0 ± 9.0	13.0 ± 7.0	54.0 ± 18.0	0.66	58.0 ± 19.0	1.0	0.1
	FM-SRP	69.0 ± 17.0	17.0 ± 10.0	11.0 ± 6.0	52.0 ± 15.0		58.0 ± 18.0		
No. sites≥5 mm	Q-SRP	69.0 ± 20.0	13.0 ± 12.0	9.0 ± 9.0	56.0 ± 20.0	0.66	60.0 ± 20.0	0.4	0.7
	FM-SRP	66.0 ± 27.0	13.0 ± 6.0	8.0 ± 5.0	53.0 ± 24.0		54.0 ± 24.0		

Mean ± sd

P– value is given for differences between Q-SRP and FM-SRP groups

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

PD = pocket depth; AL = attachment level; BOP = bleeding on probing.

Table 12. Comparison of the site-specific clinical indices before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=80 $N_{FM\text{-}SRP}$=88		Baseline	R1	p-value	R2	p– value	Change (BAS-R1)	p– value (BAS-R1)	Change (BAS-R2)	p– value (BAS-R2)	p– value (R1-R2)
PD (mm)	Q-SRP	6.2 ± 1.2	3.5 ± 1.1	0.150	3.3 ± 0.8	0.820	2.7 ± 1.3	0.002	2.9 ± 1.3	0.100	0.020
	FM-SRP	5.9 ± 1.0	3.8 ± 0.9		3.3 ± 0.8		2.1 ± 1.1		2.6 ± 1.3		
RAL (mm)	Q-SRP	14.0 ± 2.3	13.2 ± 2.3	0.870	13.0 ± 2.2	0.340	0.8 ± 1.0	0.400	1.0 ± 0.9	0.500	0.060
	FM-SRP	13.9 ± 1.8	13.2 ± 1.7		12.7 ± 1.8		0.7 ± 0.9		1.1 ± 1.0		
BOP*	Q-SRP	82.5	17.5	0.620	13.8	0.720	65.0	0.700	69.0	0.600	0.720
	FM-SRP	78.5	20.5		11.8		58.0		68.0		
Sup*	Q-SRP	61.3	3.8	1.000	1.3	0.300	57.5	0.700	60.0	0.900	1.00
	FM-SRP	64.8	3.4		4.0		61.4		59.0		
MGI	Q-SRP	2.5 ± 0.8	0.5 ± 0.5	0.970	0.2 ± 0.4	0.100	2.1 ± 1.0	0.600	2.4 ± 0.9	0.300	0.260
	FM-SRP	2.5 ± 0.6	0.5 ± 0.5		0.3 ± 0.5		2.0 ± 0.8		2.2 ± 0.8		
PI	Q-SRP	1.9 ± 0.7	0.9 ± 0.8	0.350	0.7 ± 0.7	0.460	1.1 ± 0.8	0.120	1.2 ± 0.8	0.300	0.780
	FM-SRP	2.0 ± 0.7	0.7 ± 0.6		0.6 ± 0.7		1.3 ± 0.9		1.4 ± 0.9		

Mean ± sd; *% of positive sites

P– value is given for differences between Q-SRP and FM-SRP groups

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

PD = pocket depth; RAL = relative attachment level; BOP = bleeding on probing; Sup = suppuration; MGI = modified gingival index; PI = plaque index.

When PD and RAL from the selected sites were averaged *per* patient and then compared between the two treatments the same results were found as with the site-specific analysis mentioned above (data not shown). The latter analysis was based on the assumption that sites are statistically independent units while the former used the patient as the experimental unit. In the following sections, in reference to site-specific data, these are analysed using the site as the experimental unit.

The analysis of BOP and Sup, showed no significant differences between Q-SRP and FM-SRP groups at any time point. Similarly, no significant differences in MGI and PI were seen between the treatment groups at BAS, R1 and R2.

When deep pockets (mean PD $\geq$ 7 mm) and moderate to deep pockets (mean PD < 7 mm) were considered, a significantly greater PD reduction in moderately deep pockets was seen for the Q-SRP group between BAS and R1 compared to the FM-SRP group ($p<0.01$) (Table 13). No significant differences in any other site-specific clinical index were noted in moderately deep pockets between the two treatments between BAS and R1. The analysis of the deep pockets revealed no significant differences in any clinical index between the two treatments at this time interval. This finding indicates that the greater clinical improvement at a site-specific level seen for the Q-SRP group between BAS and R1 was due to differences in PD reductions in the moderately deep pockets only between the two treatment groups. No significant differences in any clinical index were found for the moderately deep pockets between the two treatment modalities between BAS and R2. Nevertheless, the analysis of the deep pockets showed a significantly greater gain in RAL for the FM-SRP group compared to the Q-SRP group at this time interval ($p<0.05$). No other significant differences in the other clinical indices were found between the two treatment groups at R2 from BAS.

In conclusion, no statistically significant differences in the full-mouth clinical indices were seen between the two treatment groups post-therapy. Nevertheless, at a site-specific level there was a greater PD reduction for the Q-SRP group between BAS and R1 compared to the FM-SRP group, which was seen for moderately deep pockets (PD < 7 mm). In addition, a significantly greater gain in RAL was found for deep pockets (PD $\geq$ 7 mm) in the FM-SRP group at R2. However, when all the selected sites were

Table 13. Changes in the site-specific clinical indices at deep and moderately deep pockets after Q-SRP and FM-SRP.

$A_{Q\text{-}SRP}$=60 / $A_{FM\text{-}SRP}$=76 $B_{Q\text{-}SRP}$=20 / $B_{FM\text{-}SRP}$=12		A BAS-R1	p– value (BAS-R1)	A BAS-R2	p– value (BAS-R2)	B BAS-R1	p– value (BAS-R1)	B BAS-R2	p– value (BAS-R2)
ΔPD (mm)	Q-SRP	2.3 ± 1.0	0.006	2.5 ± 1.0	0.240	3.7 ± 1.7	0.580	4.3 ± 1.2	0.870
	FM-SRP	1.9 ± 0.9		2.3 ± 1.0		3.4 ± 1.0		4.4 ± 1.3	
ΔRAL (mm)	Q-SRP	0.7 ± 0.9	0.250	0.9 ± 0.9	0.780	1.1 ± 1.1	0.170	1.4 ± 0.8	0.014
	FM-SRP	0.5 ± 0.8		0.9 ± 0.9		1.6 ± 1.0		2.2 ± 0.8	
ΔBOP*	Q-SRP	70.0	0.400	75.0	0.600	55.0	0.710	70.0	1.000
	FM-SRP	63.0		70.3		66.6		66.6	
ΔSup*	Q-SRP	56.6	0.760	56.6	0.960	65.0	0.700	75.0	0.680
	FM-SRP	59.0		56.3		75.0		83.3	
ΔMGI	Q-SRP	2.0 ± 0.9	0.500	2.3 ± 0.9	0.260	2.1 ± 1.1	0.730	2.6 ± 0.9	0.830
	FM-SRP	2.0 ± 0.7		2.1 ± 0.8		2.3 ± 1.0		2.6 ± 0.8	
ΔPI	Q-SRP	1.0 ± 0.8	0.130	1.2 ± 0.9	0.200	1.3 ± 0.8	0.830	1.3 ± 0.9	0.710
	FM-SRP	1.3 ± 0.8		1.5 ± 0.9		1.1 ± 1.2		1.1 ± 1.0	

Mean ± sd; *% of positive sites

P– value is given for differences between Q-SRP and FM-SRP groups

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

PD = pocket depth; RAL = relative attachment level; BOP = bleeding on probing; Sup = suppuration; MGI = modified gingival index; PI = plaque index

A: moderately deep pockets (mean PD < 7 mm); B: deep pockets (mean PD ≥ 7 mm).

considered regardless of their initial pocket depth, no significant differences in any clinical index were detected between the treatment groups at R2 from BAS.

3.1.5 Effect of partial periodontal therapy (plaque control and root planing quadrant *per* quadrant) on the untreated sites in Q-SRP patients

At baseline, clinical indices were collected from the four selected sites. At the consecutive sessions of Q-SRP, clinical measurements were taken from the remaining not yet scaled quadrants as described earlier, in order to determine the effect of partial periodontal therapy on the untreated periodontal sites (Table 14). For sites in 2Q, a significant PD reduction of 0.5 mm occurred at visit two, two weeks after the initiation of active treatment ($p=0.005$). MGI and PI significantly improved ($p<0.05$), but there was no significant gain in RAL. BOP decreased from 90% to 75% and Sup from 60% to 50%, but these changes did not reach statistical significance.

Sites in 3Q showed a significant PD reduction of 0.4 mm at visits two and three from baseline, two and four weeks after the commencement of hygiene phase therapy and scaling of 1Q ($p<0.05$). No significant change in RAL was noted, while a significant improvement in MGI ($p<0.05$) and PI ($p<0.01$) occurred at these visits. BOP improved non-significantly from 80% to 55% and 50% ($p>0.05$) and Sup decreased significantly from 60% to 50% and finally to 25% ($p<0.05$).

Similarly, sites in 4Q showed a significant improvement in all clinical indices except for RAL and BOP, over the two, four and six weeks after the initiation of the hygiene phase of treatment. PD was reduced by 0.4 mm at visit two ($p<0.05$) and 0.6 mm at visit four from baseline ($p=0.01$). MGI improved significantly at visits three and four from baseline ($p<0.01$) and PI at all visits from baseline ($p<0.01$). Sup improved significantly at visits three and four from baseline ($p<0.01$). BOP decreased from 90% at baseline to 70%, 65% and finally to 70% at visit four, but without reaching statistical significance. Very little change in RAL was seen over a period of six weeks.

3.1.6 Patients' observations post-scaling

51 completed questionnaires were collected, 23 for the Q-SRP group and 28 for the FM-SRP group (Table 15). No significant differences in the body temperature or in the number of patients who showed elevated body temperature (> 38°C) were detected

Table 14. Effect of partial periodontal therapy on the untreated sites in Q-SRP group.

		Baseline	Visit 2	p-value (BAS-2)	Visit 3	p-value (BAS-3)	Visit 4	p-value (BAS-4)
PD(mm)	2Q	6.2 ± 0.9	5.7 ± 0.8	0.005				
	3Q	6.1 ± 1.4	5.7 ± 1.2	<0.001	5.7 ± 1.5	0.041		
	4Q	5.8 ± 1.2	5.4 ± 1.0	0.019	5.4 ± 1.1	0.066	5.2 ± 1.2	0.010
RAL(mm)	2Q	13.8 ± 2.1	13.7 ± 2.3	0.337				
	3Q	13.6 ± 2.7	13.5 ± 2.6	0.496	13.6 ± 2.6	0.727		
	4Q	13.9 ± 2.0	13.9 ± 1.8	0.793	14.0 ± 2.2	0.534	13.8 ± 1.9	0.469
BOP*	2Q	90.0	75.0	0.453				
	3Q	80.0	55.0	0.125	50.0	0.070		
	4Q	90.0	70.0	0.219	65.0	0.125	70.0	0.219
Sup*	2Q	60.0	50.0	0.625				
	3Q	60.0	50.0	0.625	25.0	0.016		
	4Q	60.0	50.0	0.625	10.0	0.006	15.0	0.004
MGI	2Q	2.7 ± 0.9	2.3 ± 0.9	0.014				
	3Q	2.6 ± 1.0	2.2 ± 0.9	0.050	1.8 ± 1.1	0.004		
	4Q	2.6 ± 0.8	2.3 ± 0.9	0.070	1.9 ± 0.9	0.003	1.8 ± 0.9	0.004
PI	2Q	1.8 ± 0.6	1.2 ± 0.8	0.004				
	3Q	2.0 ± 0.8	1.3 ± 0.9	0.006	1.0 ± 0.6	0.002		
	4Q	2.1 ± 0.6	1.5 ± 0.8	0.004	1.4 ± 0.8	0.004	1.0 ± 0.6	<0.001

1Q, 2Q, 3Q, 4Q: quadrants one, two, three and four

Mean ± sd; *% of positive sites

PD = pocket depth; RAL = relative attachment level; BOP = bleeding on probing;

Sup = suppuration; MGI = modified gingival index; PI = plaque index

P–values are given for changes from baseline.

Table 15. Patients' observations.

$N_{Q\text{-}SRP}$=23 $N_{FM\text{-}SRP}$=28	Q-SRP	FM-SRP
Pain rating (0-10)[2] Day 1	2.0 (0.0-5.5)	3.0 (0.0-9.0)
Body temperature (°C)[1] Day 1	36.7 ± 1.0	36.7 ± 0.6
Body temperature (°C)[1] Day 2	36.5 ± 0.9	36.6 ± 0.6
No. patients ≥ 38°C Day 1	3.0 (13.0%)	3.0 (11.0%)
No. patients ≥ 38°C Day 2	2.0 (5.0%)	1.0 (4.0%)
No. patients/analgesics Day 1	12.0 (52.0%)	24.0 (85.7%)
No. patients/analgesics Day 2	2.0 (8.7%)	13.0 (46.5%)
No. analgesics[2] Day 1	1.0 (0.0-6.0)	4.0 (0.0, 10.0)
No. analgesics[2] Day 2	0.0 (0.0-4.0)	0.0 (0.0-8.0)
No. patients/labial herpes	1.0 (4.0%)	2.0 (7.0%)

[1]Mean ± sd; [2]Median (min-max)

between the two treatment groups 24 hours (day one) and 48 hours (day two) after the first root planing visit ($p>0.05$).

FM-SRP patients experienced significantly greater pain than Q-SRP patients on day one ($p<0.05$). A larger number of patients in the FM-SRP group took analgesics on day one and day two compared to those in the Q-SRP group. In addition, a larger intake of analgesics was seen for the FM-SRP patients compared to the Q-SRP patients on days one and two. One patient in the Q-SRP group and two patients in the FM-SRP group presented with labial herpes post-SRP. These frequencies are too low to make any conclusions, however.

3.2 Discussion

3.2.1 Clinical findings before and after scaling and root planing

Marked improvements in all clinical indices were detected after both treatment modalities and the range of improvement in the clinical measurements is consistent with results from other studies (Ramfjord et al. 1975; Badersten et al. 1981; Listgarten et al. 1978; Badersten et al. 1984a, 1984b; Isidor et al. 1984). In the current investigation, the six-month analysis of the full-mouth clinical indices revealed a mean of 1.7 mm PD reduction, an average of 1.1 mm attachment gain and marked reduction in the number of sites with PD $\geq$ 5 mm and BOP. At the selected sites, PD decreased by 2.9 mm on average and RAL improved by 1.1 mm at six months. At this time point a marked decrease in BOP, suppuration, MGI and PI also occurred from baseline. These findings are relatively consistent with those of Cobb (1996), who demonstrated that following non-surgical periodontal therapy moderately deep and deep sites had mean PD reductions of 1.29 mm and 2.16 mm, respectively, and clinical attachment gains of 0.55 mm and 1.29 mm, respectively.

In a cross-sectional study, Quirynen et al. (1995) compared the efficacy of two treatment strategies, the one-stage full-mouth disinfection with the more common periodontal therapy of consecuitive sessions of Q-SRP. That study demonstrated that Q-SRP resulted in PD reductions of 1.7 mm at single-rooted teeth and 2.0 mm at multi-rooted teeth at two months. In a more recent study from the same research group, PD decreased by 1.2 mm at single-rooted teeth and by 1.0 mm at multi-rooted teeth for the Q-SRP group at eight months (Quirynen et al. 2000). The differences between the two

treatment strategies will be discussed in the following section. The current study showed greater PD reductions (2.9 mm) at single-rooted teeth six months after the initiation of Q-SRP treatment, compared to the findings of Quirynen et al. (1995, 2000).

The findings presented here exceed those reported by Darby et al. (2001), who showed a PD reduction of 1.5 mm and a gain in RAL of 0.4 mm in chronic periodontitis patients at approximately four to five months. One possible reason for this could be that in that study, subjects were not followed over a longer period of time (i.e. six months as in the present study). It should be stressed though that the latter study examined only four selected sites *per* patient with a PD equal to or greater than 5 mm, so comparison of clinical improvements with the present study should be made using the site level data set. The results reported in the present investigation showed a greater PD reduction and gain in AL than those by Haffajee et al. (1997a). One reason for this could be that in the mentioned study, plaque control was not optimal and no significant change in the percentage of sites exhibiting plaque was noted after treatment, resulting in an overall attachment gain of 0.11 mm and a PD reduction of 0.20 mm. In this respect, the present study showed marked reductions in plaque scores post-treatment and it has been shown that the level of oral hygiene is more critical in the PD and AL changes than the treatment modality (Lindhe et al. 1982b). Another reason may be that in the study by Haffajee et al. (1997a) the mean baseline PD was lower than that reported in the current study (3.3 mm versus 4.3 mm), which might have contributed to the lower levels of clinical improvement (Pihlstrom et al. 1983).

The present results indicated a continuous clinical improvement at three and six months. Sites were successfully treated with a single episode of mechanical debridement and no repeated deep scaling was performed up to the first three months of this study. These data confirm previous findings of Badersten et al. (1984b). In addition, a marked improvement in plaque, bleeding and suppuration scores seen after both treatment approaches paralleled marked improvements in pocket depth and attachment levels. This is in agreement with other findings which showed that attachment loss was more frequently detected in sites with high scores for plaque, bleeding, residual probing depth and suppuration than in sites with low scores (Badersten et al. 1985).

3.2.2 Comparison of clinical indices between Q-SRP and FM-SRP groups

A greater PD reduction was seen for the Q-SRP group at R1 at a site-specific level but not at R2. This could be explained solely by differences in the healing periods between the two treatments. R1 corresponded to week 13 for the Q-SRP group, i.e., six weeks after root planing the fourth quadrant, but to week seven for the FM-SRP group, i.e. six weeks after the one-day full-mouth root planing. This allowed more healing time for the sites that received Q-SRP. Patients in both treatment groups were assessed for a second time (R2) at equal time points at week 25, when no significant differences in any clinical index were evident between the two treatment groups. Indeed, FM-SRP appeared to result in a significantly greater PD reduction between R1 and R2, so that by the end of the study the FM-SRP group showed similar clinical improvement to the Q-SRP group.

When deep pockets were considered (PD $\geq$ 7 mm), the smaller PD reduction noted for the FM-SRP group between BAS and R1 compared to the Q-SRP group was due to differences in the moderately deep pockets (PD < 7 mm). This means that despite the uneven healing periods between the two treatment groups, FM-SRP resulted in similar PD reduction to Q-SRP at deep pockets. However at R2, FM-SRP resulted in a similar PD reduction but greater gain in RAL for deep pockets than Q-SRP, implying that at six months FM-SRP patients had less recession at deep pockets than Q-SRP patients.

Whole-mouth clinical indices did not reveal these differences, probably due to the dilution effect of the healthy sites. This is in agreement with Knowles et al. (1979) who reported that healing in shallow pockets has a major effect when computing patient means for change in pocket depths and masks the effects of treatment on deep pockets which are of greatest concern to the therapist.

A series of clinical trials consistently found that the one-stage full-mouth disinfection is more efficacious than consecutive sessions of quadrant root planing at two-weekly intervals (Quirynen et al. 1995; Vandekerckhove et al. 1996; Bollen et al. 1998; Mongardini et al. 1999; Quirynen et al. 2000). Greater PD reductions and attachment gains were seen for the FM-SRP group compared to the Q-SRP group, especially for deep pockets (Quirynen et al. 1995). Vandekerckhove et al. (1996) showed that for pockets $\geq$ 7 mm, a marked PD reduction of 4 mm and 3 mm and attachment gain of 3.7

mm and 1.9 mm were seen for the FM-SRP and Q-SRP groups, respectively at month eight. The present study showed the same magnitude of PD reduction in deep pockets, but the gain in RAL was notably lower for the FM-SRP group. Apart from this, at six months the only significant difference between the two treatment groups was the greater attachment gain seen only for deep pockets in the FM-SRP group. This finding was despite the fact that full-mouth root planing was completed in 12 hours rather than the 24 hours seen in other studies (Quirynen et al. 1995; Vandekerckhove et al. 1996), and that the patients in the FM-SRP group were seen at equal time points and received equal amounts of oral hygiene instructions to those that received Q-SRP. This resulted in similar plaque indices between the two treatment groups throughout the study, which is in disagreement with findings of Vandekerckhove et al. (1996), who showed higher plaque indices for the one-stage full-mouth disinfection group after the first month possibly due to lack of frequent sessions of oral hygiene reinforcement.

It must be noted, that in the first investigations by Quirynen et al. (1995) and Vandekerckhove (1996) the baseline readings collected from the single-rooted teeth were not comparable between the two treatment groups, since there was a 0.8 mm difference between them, with the deeper pockets seen for the one-stage full-mouth disinfection group. In addition, probing depth was recorded immediately after root planing, possibly resulting in an overestimation of the disease severity. In a later study by Quirynen et al. (2000), three groups of patients were examined, but two of the groups participated and received treatment in an earlier trial by the same investigators (Quirynen et al. 1999, Mongardini et al. 1999). The third group of patients was recruited later and although the assessment of patients in this group started in the middle of the previous study, this could have resulted in biased data collection.

Several reasons could account for the differences between the current study and the trials described above. In the latter studies clinical measurements were collected from quadrant one only, even before Q-SRP therapy was completed. Therefore, the final clinical changes seen in the Q-SRP group may not have been revealed at this stage. It was interesting to note that no further PD reduction occurred in single-rooted teeth of the Q-SRP patients after two months, when root planing of the whole dentition was completed (Quirynen et al. 1995). In the current study, patients were reassessed six weeks after the completion of active treatment and then six months after the initiation of

treatment, and a continuous clinical improvement was evident during the monitoring period, reaching a plateau at six months. This finding is consistent with previous results (Badersten et al. 1984a). The disparity between the present investigation and the studies of Quirynen et al. (1995, 2000) is mainly due to differences in the clinical outcome seen in the Q-SRP group. In the current study, PD reduction following Q-SRP treatment was remarkably greater than that found in the quoted studies.

Another difference in the methodology between the current and the study of Quirynen et al. (1995) is that this investigation examined the clinical outcome of conventional periodontal therapy consisting of quadrant or full-mouth scaling and root planing and oral hygiene instructions with no adjunctive use of antiseptics. The clinical results presented in the current study are not inferior to those reported by the other studies that used antimicrobial agents adjunctive to mechanical debridement (Listgarten et al. 1978; Haffajee et al. 1988b; Mombelli et al. 1996). Data from other studies agree that chlorhexidine does not augment the beneficial outcome of periodontal therapy (Braatz et al. 1985; MacAlpine et al. 1985; Wennström et al. 1987a, 1987b), and when this does occur, it is a transient phenomenon rather than a long-term effect (Lander et al. 1986; Oosterwaal et al. 1991). Studies where there is lack of meticulous plaque control and where the root planing is not thorough, tend to present the most promising results for the use of chlorhexidine alone, or in conjunction with root planing.

Bollen et al. (1998) showed that the extended and prolonged use of chlorhexidine in the one-stage full-mouth disinfection resulted in additional improvements both clinically and microbiologically, to those reported in previous studies (Quirynen et al. 1995). Nevertheless, this finding is in disparity with more recent data from the same research group, which showed that no significant differences in any clinical index existed between patients who received full-mouth root planing, with or without the use of chlorhexidine, and that the clinical outcome of these treatments was superior to that of quadrant root planing at two-weekly intervals (Quirynen et al. 2000). The authors concluded that the role of chlorhexidine in the beneficial effects of the one-stage full-mouth root planing is not critical, implying that a host-induced effect could contribute to the superior clinical outcome seen after this treatment strategy. These considerations will be discussed in Chapter four.

3.2.3 Effect of partial periodontal therapy on the untreated sites in Q-SRP patients

The current study reported that partial periodontal therapy, which occurred in the course of quadrant root planing at two-weekly intervals, resulted in improved clinical conditions in the remaining untreated quadrants, in terms of pocket depth, suppuration, gingival and plaque indices reductions. This finding could be the result of improved plaque control in highly motivated periodontitis patients and / or host-induced effects during the active phase of treatment and / or a "Hawthorne effect" following recruitement into the study. The speculation on host-induced benefits will be discussed in detail in Chapter four. The Hawthorne effect was first described in a series of experimental studies of employees, at the Western Electric Company's Hawthorne (Illinois) plant between 1927 and 1932 (Roethlisberger and Dickson, 1939). It was concluded that the employees' awareness of their participation in a research study affected their behaviour (i.e. to increase production) (Mayo, 1933). It is also possible that during the course of active treatment, patients notice improvements in the scaled quadrants in contrast to the untreated ones and this reinforces them to practise improved oral hygiene measures.

During the monitoring period of six weeks, PD reduction of 0.4-0.6 mm occurred but minimum improvement in RAL and bleeding scores occurred, which is in accordance with findings of Badersten et al. (1984a). In their study, clinical improvements were seen following plaque control measures, but these reductions were limited in comparison to those found after instrumentation. The authors suggested that these changes were essentially due to gingival recession. The current findings confirm this notion, by showing significant reductions in PD and MGI, but not in RAL following the initiation of plaque control measures.

Several studies showed that the institution of plaque control measures affects the composition of the microbial flora subgingivally, as well as the clinical indices (Smulow et al. 1983; Dahlén et al. 1992; Katsanoulas et al. 1992; McNabb et al. 1992; Hellström et al. 1996; Haffajee et al. 2001a; 2001b). Nevertheless, it is doubtful whether supragingival plaque control has an effect on the subgingival microflora in the apical aspects of deep pockets (Kho et al. 1985; Beltrami et al. 1987; Loos et al. 1988). The data presented in the current study are consistent with other findings which

have shown that the clinical outcome of supragingival control alone is not as favourable as that seen after subgingival instrumentation (Badersten et al. 1984a; Kaldahl et al. 1996a; Westfelt et al. 1998).

3.2.4 Patients' observations

The completed questionnaires revealed that FM-SRP resulted in significantly higher pain rating than Q-SRP. No significant differences in body temperature were seen between the two treatment groups 24 and 48 hours after the first scaling session. Nevertheless, it was of interest to see that almost the same number of Q-SRP and FM-SRP patients had a body temperature $\geq$ 38°C at 24 hours and 48 hours after instrumentation. However, a significantly higher percentage of FM-SRP patients took analgesics 24 and 48 hours after debridement and, since some of the analgesics have antipyretic properties, this may contribute to the same number of Q-SRP and FM-SRP patients having increased body temperature. One Q-SRP patient and two FM-SRP patients presented with labial herpes post-scaling, which could be due to trauma from the procedures or to a host-induced effect after thorough and deep root planing. The host response to treatment will be discussed with the humoral immunological results (Chapter four).

The fact that higher pain scores and higher intake of analgesics was demonstrated for the FM-SRP group compared to the Q-SRP group agrees with the findings of another study (Quirynen et al. 2000). It should be borne in mind that the FM-SRP treatment protocol in the present investigation is more comparable to that of the one-stage full-mouth root planing without the use of chlorhexidine in the study of Quirynen et al. (2000). Nevertheless, this study showed that no Q-SRP patients had elevated body temperature on day one and / or labial herpes, which is in contrast to our findings.

However, body-temperature was self-recorded and although precise instructions for its measurement were given, significant individual variability existed with respect to usage of different makes of thermometers and differences in the body sites used or the time of the day that patients measured their body temperature. Taking into account these technical difficulties, as well as an inter-subject variability regarding the following factors: pain threshold, tolerance to dental procedures, and the fact that some analgesics have anti-pyretic properties, this investigation suggests that the completed

questionnaires are only indicative of post-treatment complications and therefore no attempt was made to relate these data to experimental findings.

CHAPTER 4

EFFECT OF SCALING AND ROOT PLANING ON IMMUNOLOGICAL PARAMETERS

4. Effect of scaling and root planing on immunological parameters

4.1 Results

4.1.1 Immunological responses in Q-SRP patients

4.1.1.1 Serum IgG antibody titres

In general, there was a decrease in serum IgG titres (expressed as EU) against the five bacteria following treatment (Table 16). For *P. gingivalis*, there was a statistically significant decrease in the antibody levels at R2 ($p<0.005$). For *P. intermedia* and *T. denticola* a reduction in serum antibody titres was noted at R2, but these findings just failed to reach statistical significance ($p=0.05$ for *P. intermedia*, $p=0.052$ for *T. denticola*). No significant reductions in antibody levels were noted for *A. actinomycetemcomitans* and *B. forsythus* following treatment.

4.1.1.2 GCF volume and GCF IgG antibody titres

GCF IgG titres (expressed as EU / 30 sec) decreased significantly post-treatment for *A. actinomycetemcomitans* at R2 ($p<0.05$) and for *T. denticola* at R1 ($p<0.01$) and R2 ($p=0.01$) (Table 17). No significant changes were noted after treatment for the other suspected periodontal pathogens, although *P. gingivalis* changes were close to statistical significance. Table 18 shows the changes of GCF volume (expressed as µl / 30 sec) with treatment. A significant reduction was found at R1 and R2 ($p<0.001$).

4.1.1.3 IgG antibody avidity

Despite the general trend for IgG avidity (expressed as M at ID_{50}) to increase six months post-treatment, changes did not reach statistical significance (Table 19). Therefore, Q-SRP resulted in antibodies of similar avidity after treatment.

4.1.2 Immunological responses in FM-SRP patients

4.1.2.1 Serum IgG antibody titres

In a similar way to Q-SRP, one-day FM-SRP resulted in significantly lower serum IgG antibody titres (EU) after treatment for the majority of the tested bacteria (Table 20). Nevertheless, no significant decreases were noted for *P. gingivalis* and *B. forsythus* post-treatment. IgG antibody titres to *A. actinomycetemcomitans*, significantly decreased at R2 ($p<0.05$) and antibody levels to *P. intermedia* significantly decreased at R1 ($p=0.005$). Median IgG titres against *T. denticola*, were significantly reduced at R1 ($p=0.001$) and R2 ($p<0.01$).

Table 16. Changes in serum IgG antibody titres for the Q-SRP group at R1 and R2.

N=20	Baseline	R1	R2	Change (BAS-R1)	p– value	Change (BAS-R2)	p– value
P.g.	149 (42, 809)	81 (29, 843)	72 (21, 535)	11 (-20, 93)	0.300	49 (2, 387)	0.004
A.a.	63 (18, 211)	52 (20, 394)	35 (21, 175)	4 (-16, 54)	0.320	8 (-9, 36)	0.212
P.i.	144 (64, 403)	131 (50, 694)	101 (47, 399)	6 (-67, 42)	0.990	30 (-13, 131)	0.050
T.d.	13 (5, 75)	15 (4, 59)	13 (2, 57)	1 (-19, 3)	0.970	3 (0, 13)	0.052
B.f.	33 (17, 1156)	29 (14, 1007)	37 (13, 542)	-1 (-7, 71)	1.000	6 (-1, 60)	0.093

IgG titres are expressed as ELISA units (EU)

Median (Q1, Q3)

P.g.: P. gingivalis; *A.a.: A.. actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 17. Changes in GCF IgG antibody titres for the Q-SRP group at R1 and R2.

N=80	Baseline	R1	R2	Change (BAS-R1)	p– value	Change (BAS-R2)	p– value
P.g.	21.0 ± 14.0	1919.0 ± 1238.0	16.0 ± 6.0	-1898.0 ± 1239.0	0.24	4.2 ± 15.0	0.06
A.a.	5221.0 ± 3742.0	122.0 ± 117.0	12.0 ± 9.0	5099.0 ± 3744.0	0.29	5209.0 ± 3742.0	0.02
P.i.	3.0 ± 0.7	2.0 ± 0.3	2.0 ± 0.2	0.8 ± 0.7	0.86	0.8 ± 0.7	0.53
T.d.	4.3 ± 1.0	1.0 ± 0.2	1.7 ± 0.4	3.3 ± 1.3	<0.01	2.6 ± 1.2	0.01
B.f.	2.4 ± 0.8	2.0 ± 0.6	2.3 ± 1.0	0.5 ± 0.7	0.21	0.2 ± 1.2	0.51

GCF IgG titres are expressed as EU/30sec

Mean ± SEM

P.g.: P. gingivalis; *A.a.: A.. actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 18. Comparison of GCF volume before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=80 $N_{FM\text{-}SRP}$=88	Baseline	p-value[2]	R1	R2	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
Q-SRP	0.5 ± 0.3	0.190	0.2 ± 0.2	0.2 ± 0.1	0.3 ± 0.3	<0.001	0.490	0.3 ± 0.4	<0.001	0.170
FM-SRP	0.4 ± 0.3		0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.3	<0.001		0.2 ± 0.3	<0.001	

GCF volume is expressed as µl/30 sec

Mean ± sd

[1]P-value is given for changes before/after SRP in each group

[2] P-value is given for differences between Q-SRP and FM-SRP groups

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 19. Changes in IgG antibody avidity for the Q-SRP group at R1 and R2.

N=20	Baseline	R1	R2	Change (BAS-R1)	p– value	Change (BAS-R2)	p– value
P.g.	0.46 (0.30, 0.60)	0.45 (0.30, 0.60)	0.49 (0.30, 0.90)	-0.02 (-0.10, 0.10)	0.60	-0.06 (-0.30, 0.06)	0.10
A.a.	0.38 (0.30, 0.60)	0.40 (0.30, 0.50)	0.42 (0.30, 0.70)	0.02 (-0.10, 0.10)	0.40	-0.04 (-0.2, 0.05)	0.08
P.i.	0.84 (0.60, 1.00)	0.72 (0.60, 0.90)	0.86 (0.70, 1.10)	0.08 (-0.10, 0.30)	0.15	0.005 (-0.20, 0.08)	0.90
T.d.	0.30 (0.20, 0.40)	0.31 (0.30, 0.50)	0.33 (0.30, 0.40)	-0.04 (-0.20, 0.02)	0.08	-0.03 (-0.08, 0.04)	0.20
B.f.	0.37 (0.20, 0.70)	0.41 (0.30, 0.60)	0.38 (0.30, 0.70)	0.00 (-0.20, 0.10)	0.65	0.00 (-0.09, 0.05)	0.59

IgG avidity is expressed as molarity (M) at ID_{50}

Median (Q1, Q3)

P.g.: P. gingivalis; *A.a.: A.. actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 20. Changes in serum IgG antibody titres for the FM-SRP group at R1 and R2.

N=22	Baseline	R1	R2	Change (BAS-R1)	p– value	Change (BAS-R2)	p– value
P.g.	79 (30, 449)	80.0 (27, 456)	119 (29, 184)	2 (-25, 61)	0.580	14 (-33, 371)	0.220
A.a.	52 (12, 1069)	36.0 (14, 430)	51 (16, 445)	4 (-2, 97)	0.095	10 (-2, 719)	0.028
P.i.	298 (56, 818)	245 (76, 461)	335 (135, 475)	53 (-4, 207)	0.005	26 (-44, 298)	0.140
T.d.	18 (8, 59)	11 (6, 26)	15 (8, 26)	7 (0.8, 34)	0.001	6 (1, 35)	0.007
B.f.	32 (13, 858)	33.0 (10, 411)	34 (15, 304)	6 (-6, 72)	0.180	2 (-5, 462)	0.077

Serum IgG titres are expressed as ELISA units (EU)

Median (Q1, Q3)

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

4.1.2.2 GCF volume and GCF IgG antibody titres

In general, GCF IgG titres (EU / 30 sec) appeared to have low values with large subject variability (Table 21). A significant decrease in IgG titres against *T. denticola* was noted at R1 ($p<0.05$) and R2 ($p<0.001$) and against *P. intermedia* at R2 ($p<0.05$). When *B. forsythus* was examined, a small but statistically significant increase was noted at R1 ($p<0.005$). Table 18 shows that GCF volume (µl / 30 sec) significantly decreased at R1 and R2 ($p<0.001$).

4.1.2.3 IgG antibody avidity

IgG avidity (M at ID_{50}) tended to increase post-treatment for the majority of the organisms but this observation was statistically significant for antibodies against *A. actinomycetemcomitans* only (Table 22). For *A. actinomycetemcomitans* there was an increase from 0.44 (0.36, 0.60) M at baseline to 0.55 (0.36, 0.80) M at R2 ($p<0.02$).

4.1.3 Comparison of immunological parameters in Q-SRP and FM-SRP groups

Tables 23 and 24 show that there were no statistically significant differences in serum IgG antibody titres (EU) and avidity (M at ID_{50}) between the two treatments at BAS and at R1 and R2. However, when the changes at R1 and R2 from BAS were examined, there was a significantly greater reduction in serum IgG titres against *P. intermedia* and *T. denticola* at R1 for the FM-SRP group ($p<0.05$) (Table 23). No significant differences in changes of IgG avidity with treatment were seen between Q-SRP and FM-SRP groups (Table 24).

Table 25 shows that no significant differences were detected in GCF IgG titres (EU / 30 sec) between the treatment groups at baseline. However, higher GCF IgG titres against *A. actinomycetemcomitans* and *P. intermedia* were noted at R2 for the FM-SRP group compared to the Q-SRP group ($p=0.05$ for *A. actinomycetemcomitans*, $p<0.01$ for *P. intermedia*). In addition, GGF antibody levels against *T. denticola* were higher at R1 for the FM-SRP group compared to the Q-SRP group ($p<0.05$). No significant differences in GCF volume (µl / 30 sec) were detected between the Q-SRP and FM-SRP groups at baseline and following treatment (Table 18).

Table 21. Changes in GCF IgG antibody titres for the FM-SRP group at R1 and R2.

N=88	Baseline	R1	R2	Change (BAS-R1)	p– value	Change (BAS-R2)	p– value
P.g.	15.0 ± 11.0	4.0 ± 0.6	15.0 ± 11.0	11.4 ± 11.0	0.360	3.0 ± 17.0	0.310
A.a.	342.0 ± 137.0	1535.0 ± 1099.0	170.3 ± 98.0	-1151.0 ± 1062.0	0.400	238.0 ± 140.0	0.400
P.i.	4.3 ± 2.0	2.4 ± 0.3	2.6 ± 0.2	2.0 ± 2.0	0.150	2.3 ± 2.6	0.040
T.d.	8.0 ± 3.0	2.1 ± 0.3	2.3 ± 0.7	6.0 ± 3.1	0.017	6.6 ± 3.7	<0.001
B.f.	1.5 ± 0.6	2.3 ± 0.8	1.1 ± 0.1	-0.9 ± 1.0	0.004	0.6 ± 0.8	0.160

GCF IgG titres are expressed as EU/30 sec

Mean ± SEM

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 22. Changes in IgG antibody avidity for the FM-SRP group at R1 and R2.

N=22	Baseline	R1	R2	Change (BAS-R1)	p– value	Change (BAS-R2)	p– value
P.g.	0.49 (0.30, 0.60)	0.58 (0.30, 0.70)	0.57 (0.30, 0.70)	-0.03 (-0.10, 0.12)	0.88	-0.03 (-0.08, 0.15)	0.53
A.a.	0.44 (0.40, 0.60)	0.43 (0.40, 0.70)	0.55 (0.40, 0.80)	-0.02 (-0.20, 0.05)	0.26	-0.09 (-0.30, 0.02)	<0.02
P.i.	0.84 (0.70, 1.10)	0.85 (0.70, 1.20)	0.85 (0.70, 1.20)	0.01 (-0.10, 0.11)	0.82	0.06 (-0.06, 0.14)	0.33
T.d.	0.36 (0.30, 0.50)	0.45 (0.30, 0.80)	0.40 (0.30, 0.50)	-0.10 (-0.30, 0.07)	0.07	0.00 (-0.10, 0.06)	0.63
B.f.	0.46 (0.30, 0.60)	0.49 (0.30, 0.60)	0.49 (0.30, 0.90)	0.02 (-0.10, 0.10)	0.95	-0.05 (-0.30, 0.05)	0.09

IgG avidity is expressed as molarity (M) at ID_{50}

Median (Q1, Q3)

P.g.: P. gingivalis; *A.a.: A.. actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 23. Comparison of serum IgG antibody titres before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=20 $N_{FM\text{-}SRP}$=22	Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g. Q-SRP	149 (42, 809)	0.50	81 (29, 843)	0.91	72 (21, 535)	0.87	11 (-20, 93)	0.68	49 (2, 387)	0.29
FM-SRP	79 (30, 449)		80 (27, 456)		119 (29, 184)		2 (-25, 61)		14 (-33, 371)	
A.a. Q-SRP	63 (18, 211)	1.00	52 (20, 394)	0.74	35 (21, 175)	0.68	4 (-16, 54)	0.91	8 (-9, 36)	0.38
FM-SRP	52 (12, 1069)		36 (14, 430)		51 (16, 445)		4 (-2, 97)		10 (-2, 719)	
P.i. Q-SRP	144 (64, 403)	0.30	131 (50, 694)	0.62	101 (47, 399)	0.07	6 (-67, 42)	<0.03	30 (-13, 131)	0.82
FM-SRP	298 (56, 818)		245 (76, 461)		335 (135, 475)		53 (-4, 207)		26 (-44, 298)	
T.d. Q-SRP	13 (5, 75)	0.60	15 (4, 59)	0.62	13 (2, 57)	1.00	1 (-19, 3)	0.01	3 (0, 13)	0.26
FM-SRP	18 (8, 59)		11 (6, 26)		15 (8, 26)		7 (1, 34)		6 (1, 35)	
B.f. Q-SRP	33 (17, 1156)	0.80	29 (14, 1007)	0.29	37 (13, 542)	0.17	-1 (-7, 71)	0.55	6 (-1, 60)	0.96
FM-SRP	32 (13, 858)		33 (10, 411)		34 (15, 304)		6 (-6, 72)		2 (-5, 462)	

Serum IgG titres are expressed as ELISA units (EU)

Median (Q1, Q3)

P-values are given for differences between Q-SRP and FM-SRP groups

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 24. Comparison of IgG antibody avidity before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=20 $N_{FM\text{-}SRP}$=22	Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g. Q-SRP	0.46 (0.30, 0.60)	0.50	0.45 (0.30, 0.60)	0.47	0.49 (0.30, 0.90)	0.91	-0.02 (-0.10, 0.08)	0.76	-0.06 (-0.30, 0.06)	0.51
FM-SRP	0.49 (0.30, 0.60)		0.58 (0.30, 0.70)		0.57 (0.30, 0.70)		-0.03 (-0.10, 0.12)		-0.03 (-0.08, 0.15)	
A.a. Q-SRP	0.38 (0.30, 0.60)	0.30	0.4 (0.30, 0.50)	0.10	0.42 (0.30, 0.70)	0.37	0.02 (-0.06, 0.10)	0.13	-0.04 (-0.02, 0.05)	0.31
FM-SRP	0.44 (0.40, 0.60)		0.43 (0.40, 0.70)		0.55 (0.40, 0.80)		-0.02 (-0.20, 0.05)		-0.09 (-0.30, 0.02)	
P.i. Q-SRP	0.84 (0.60, 1.00)	0.60	0.72 (0.60, 0.90)	0.17	0.86 (0.70, 1.10)	0.74	0.08 (-0.09, 0.30)	0.34	0.005 (-0.20, 0.08)	0.33
FM-SRP	0.84 (0.70, 1.10)		0.85 (0.70, 1.20)		0.85 (0.70, 1.20)		0.005 (0.60, 0.10)		0.06 (-0.06, 0.14)	
T.d. Q-SRP	0.30 (0.20, 0.40)	0.10	0.31 (0.30, 0.50)	0.10	0.33 (0.30, 0.40)	0.12	-0.04 (-0.20, 0.02)	0.97	-0.03 (-0.08, 0.04)	0.78
FM-SRP	0.36 (0.30, 0.50)		0.45 (0.40, 0.80)		0.4 (0.30, 0.50)		-0.10 (-0.30, 0.07)		0.00 (-0.10, 0.06)	
B.f. Q-SRP	0.37 (0.20, 0.70)	0.20	0.41 (0.30, 0.60)	0.29	0.38 (0.30, 0.70)	0.17	0.00 (-0.15, 0.10)	0.77	0.00 (-0.10, 0.05)	0.56
FM-SRP	0.46 (0.30, 0.60)		0.49 (0.30, 0.60)		0.49 (0.30, 0.90)		0.015 (-0.09, 0.10)		-0.05 (-0.30, 0.05)	

IgG avidity is expressed as molarity (M) at ID_{50}

Median (Q1, Q3)

P-values are given for differences between Q-SRP and FM-SRP groups

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 25. Comparison of GCF IgG antibody titres before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=80 $N_{FM\text{-}SRP}$=88		Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g.	Q-SRP	21.0 ± 14.0	0.990	1919.0 ± 1238.0	0.450	16.0 ± 6.0	0.860	-1898.0 ± 1239.0	0.890	4.2 ± 15.0	0.350
	FM-SRP	15.0 ± 11.0		4.0 ± 0.6		15.0 ± 11.0		11.4 ± 11.0		3.0 ± 17.0	
A.a.	Q-SRP	5221.0 ± 3742.0	0.970	122.0 ± 117.0	0.240	12.0 ± 9.0	0.050	5099.0 ± 3744.0	0.890	5209 ± 3742.0	0.270
	FM-SRP	342.0 ± 137.0		1535.0 ± 1099.0		170.0 ± 98.0		-1151.0 ± 1062.0		238.0 ± 140.0	
P.i.	Q-SRP	2.7 ± 0.7	0.340	2.0 ± 0.3	0.065	2.0 ± 2.0	0.007	0.8 ± 0.7	0.300	0.8 ± 0.7	0.120
	FM-SRP	4.3 ± 2.1		2.4 ± 0.3		2.6 ± 2.0		1.9 ± 2.2		2.3 ± 2.6	
T.d.	Q-SRP	4.3 ± 1.3	0.170	1.0 ± 0.2	0.012	1.7 ± 0.4	0.880	3.3 ± 1.3	0.430	2.6 ± 1.2	0.410
	FM-SRP	8.1 ± 3.0		2.1 ± 0.3		2.3 ± 0.7		6.0 ± 3.0		6.6 ± 3.7	
B.f.	Q-SRP	2.4 ± 0.8	0.340	1.9 ± 0.6	0.820	2.3 ± 1.0	0.080	0.5 ± 0.7	0.450	0.2 ± 1.2	0.070
	FM-SRP	1.5 ± 0.7		2.3 ± 0.8		1.1 ± 0.1		-0.9 ± 1.0		0.6 ± 0.8	

GCF IgG titres are expressed as EU/30sec

Mean ± SEM

P-values are given for differences between Q-SRP and FM-SRP groups

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

4.1.4 Correlation between GCF antibody titres and GCF volume

At baseline, GCF IgG titres (EU / 30 sec) were positively correlated with GCF volume (µl / 30 sec), indicating that the higher the GCF volume, the higher the GCF IgG antibody levels are (data not shown). This finding was statistically significant for *A. actinomycetemcomitans*, *T. denticola*, and *B. forsythus*. Correlation coefficient and p-value for each of the bacteria tested were as follows: 0.070 and 0.330 for *P. gingivalis*; 0.220 and 0.004 for *A. actinomycetemcomitans*; 0.080 and 0.300 for *P. intermedia*; 0.220 and 0.004 for *T. denticola*; 0.162 and 0.036 for *B. forsythus*.

At six months (R2), GCF volume was positively correlated with GCF antibody levels to *A. actinomycetemcomitans* (R=0.13, p=0.10), *T. denticola* (R=0.11, p=0.17), and *B. forsythus* (R=0.08, p=0.35), but this observation failed to reach statistical significance. However, GCF volume was negatively correlated with GCF antibody titres to *P. gingivalis* (R=-0.2, p<0.02) and to *P. intermedia* (R=-0.04, p=0.60) at R2, and this finding was statistically significant for the former organism.

4.1.5 Serum IgG antibody dynamics following periodontal treatment

The dynamics of the systemic IgG antibody response over the course of treatment (six visits in total) was determined by analysing the changes in serum IgG titres and avidity from baseline to each follow up visit i.e. BAS to visit two, BAS to visit three, BAS to visit four, BAS to visit five and BAS to visit six. These visits were carried out at equal time points for both treatment groups. In order to determine differences in the immunological parameters (titres and avidity) between the Q-SRP group and the FM-SRP group over the course of treatment, the changes between BAS and each follow up visit, and also the findings of each visit with no reference to baseline were compared.

4.1.5.1 Effect of Q-SRP on the humoral immune response dynamics

The median serum IgG antibody levels (EU) at each visit and the p-values of the changes from baseline are shown in Table 26. In general, serum IgG antibody titres did not appear to increase during the active phase of Q-SRP treatment with reference to baseline. A significant reduction in IgG titres to *P. gingivalis* was found between BAS and visit six (p<0.005). Serum IgG titres to *A. actinomycetemcomitans* showed a significant reduction between BAS and visit three (p<0.05). Similarly, serum antibody titres to *P. intermedia*, decreased between BAS and visit six, but this finding just failed

Table 26. Serum IgG antibody titres over six visits of treatment for Q-SRP and FM-SRP groups.

$N_{Q\text{-}SRP}$=20 $N_{FM\text{-}SRP}$=22		Baseline	Visit 2	p-value BAS-2	Visit 3	p-value BAS-3	Visit 4	p-value BAS-4	Visit 5	p-value BAS-5	Visit 6	p-value BAS-6
P.g.	Q-SRP	149 (42, 809)	98 (21, 546)	0.320	88 (37, 746)	0.940	108 (30, 458)	0.190	81 (29, 843)	0.310	72 (21, 535)	0.004
	FM-SRP	79 (30, 449)	83 (45, 334)	0.450	115 (40, 747)	0.570	80 (27, 456)	0.580	65 (25, 370)	0.020	119 (29, 184)	0.220
A.a.	Q-SRP	63 (18, 211)	40 (18, 340)	0.100	37 (15, 120)	0.020	38 (18, 358)	0.220	52 (20, 394)	0.320	35 (21, 175)	0.210
	FM-SRP	52 (12, 1069)	43 (19, 345)	0.002	66 (18, 408)	0.480	36 (14, 430)	0.090	39 (18, 657)	0.550	51 (16, 445)	<0.030
P.i.	Q-SRP	144 (64, 403)	95 (41, 428)	0.230	102 (51, 540)	0.280	98 (48, 518)	0.130	131 (50, 694)	0.980	101 (47, 399)	0.050
	FM-SRP	298 (56, 818)	329 (112, 534)	0.310	273 (135, 600)	0.210	245 (76, 461)	0.005	202 (94, 506)	0.048	335 (135, 475)	0.140
T.d.	Q-SRP	13 (5, 75)	11 (3, 34)	0.240	12 (3, 28)	0.040	15 (3, 68)	0.980	15 (4, 59)	0.960	13 (2, 57)	0.052
	FM-SRP	18 (8, 59)	16 (7, 37)	0.090	14 (6, 43)	0.040	11 (6, 26)	0.001	12 (7, 35)	0.030	15 (8, 26)	0.007
B.f.	Q-SRP	33 (17, 1156)	50 (11, 345)	0.730	30 (15, 530)	0.080	29 (16, 931)	0.190	29 (14, 1007)	1.000	37 (13, 542)	0.090
	FM-SRP	32 (13, 858)	38 (12, 808)	0.540	30 (22, 340)	0.850	33 (10, 411)	0.180	47 (16, 343)	0.720	34 (15, 304)	0.080

Serum IgG titres are expressed as ELISA units (EU)

Median (Q1, Q3)

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing.

to reach statistical significance (p=0.05). For *T. denticola*, serum antibody levels decreased at visit three ($p<0.05$) and visit six from baseline (p=0.052).

The analysis of the IgG avidity (M at ID_{50}) over the six visits for the Q-SRP group found that for *P. gingivalis*, a significant increase was noted between BAS and visit four ($p<0.05$) and for *A. actinomycetemcomitans* between BAS and visits three and four ($p<0.05$) (Table 27). IgG avidity to *T. denticola* increased between BAS and each session of Q-SRP, significantly at visits two ($p<0.005$), three ($p<0.05$) and four ($p<0.01$) from BAS.

4.1.5.2 Effect of FM-SRP on the humoral immune response dynamics

Table 26 shows the serum IgG antibody levels (EU) at each visit during treatment and also depicts the p-values of the changes in the serum antibody titres at each visit from baseline. A significant decrease in IgG titres to *P. gingivalis* was noted between BAS and visit five ($p<0.05$) and to *A. actinomycetemcomitans* at visits two ($p<0.005$) and six ($p<0.05$) from BAS. The IgG levels to *P. intermedia* decreased at visits four (p=0.005) and five ($p<0.05$) from BAS, and a significant decrease at visits three, four, five and six from BAS was found for *T. denticola* ($p<0.05$).

IgG avidity (M at ID_{50}) and the p-values of the changes from baseline are shown in Table 27. An increase in IgG avidity to *A. actinomycetemcomitans* was found at visits two ($p<0.05$), three ($p<0.005$) and visit six ($p<0.05$) from BAS, and to *B. forsythus* at visit three from BAS ($p<0.01$).

4.1.5.3 Humoral immune response dynamics after Q-SRP and FM-SRP

No significant differences were detected in the changes of serum IgG antibody titres (EU) against *P. gingivalis*, between Q-SRP and FM-SRP groups at any of the five time points from baseline (Table 28). This was a consistent finding with *A. actinomycetemcomitans*, *P. intermedia*, and *B. forsythus*. However, when *T. denticola* was examined, a significant difference was seen in the changes of IgG antibody levels between BAS and visit four (p=0.01), with a greater reduction in the IgG antibody levels for the FM-SRP group compared to the Q-SRP group. When IgG antibody levels were compared between the two treatment groups at each visit with no reference to baseline, no significant differences were detected against any of the tested bacteria (Table 28).

Table 27. IgG antibody avidity over six visits of treatment for Q-SRP and FM-SRP groups.

$N_{Q\text{-}SRP}$=20 $N_{FM\text{-}SRP}$=22	Baseline	Visit 2	p-value BAS-2	Visit 3	p-value BAS-3	Visit 4	p-value BAS-4	Visit 5	p-value BAS-5	Visit 6	p-value BAS-6
P.g. Q-SRP	0.46 (0.30, 0.60)	0.48 (0.30, 0.70)	0.090	0.47 (0.40, 0.80)	0.090	0.44 (0.30, 0.70)	<0.040	0.45 (0.30, 0.60)	0.600	0.49 (0.30, 0.90)	0.100
FM-SRP	0.49 (0.30, 0.60)	0.49 (0.30, 0.80)	0.320	0.54 (0.40, 0.80)	0.400	0.58 (0.30, 0.70)	0.880	0.51 (0.30, 0.70)	0.270	0.57 (0.30, 0.70)	0.530
A.a. Q-SRP	0.38 (0.30, 0.60)	0.39 (0.30, 0.60)	0.170	0.41 (0.40, 0.60)	0.020	0.50 (0.30, 0.60)	0.025	0.40 (0.30, 0.50)	0.420	0.42 (0.30, 0.70)	0.080
FM-SRP	0.44 (0.40, 0.60)	0.56 (0.40, 0.80)	0.048	0.54 (0.40, 0.80)	0.004	0.43 (0.40, 0.70)	0.260	0.44 (0.40, 0.70)	0.640	0.55 (0.40, 0.80)	0.015
P.i. Q-SRP	0.84 (0.60, 1.00)	0.90 (0.70, 1.20)	0.057	0.97 (0.70, 1.20)	0.079	0.83 (0.60, 1.00)	0.550	0.72 (0.60, 0.90)	0.150	0.86 (0.70, 1.10)	0.920
FM-SRP	0.84 (0.70, 1.10)	0.95 (0.70, 1.30)	0.510	0.96 (0.70, 1.30)	0.130	0.85 (0.70, 1.20)	0.820	0.83 (0.70, 1.20)	0.410	0.85 (0.70, 1.20)	0.320
T.d. Q-SRP	0.30 (0.20, 0.40)	0.40 (0.30, 0.70)	0.003	0.35 (0.20, 0.70)	0.028	0.36 (0.30, 0.60)	0.009	0.31 (0.30, 0.50)	0.080	0.33 (0.30, 0.40)	0.210
FM-SRP	0.36 (0.30, 0.50)	0.32 (0.30, 0.50)	0.740	0.45 (0.30, 0.50)	0.850	0.45 (0.30, 0.80)	0.070	0.38 (0.30, 0.60)	0.350	0.40 (0.30 0.50)	0.630
B.f. Q-SRP	0.37 (0.20, 0.60)	0.43 (0.30, 0.70)	0.080	0.38 (0.30, 0.70)	0.100	0.37 (0.30, 0.60)	0.750	0.41 (0.30, 0.60)	0.650	0.38 (0.30, 0.70)	0.590
FM-SRP	0.46 (0.30, 0.60)	0.49 (0.40, 0.70)	0.510	0.62 (0.40 0.80)	0.009	0.49 (0.30, 0.60)	0.940	0.52 (0.30, 0.60)	0.350	0.49 (0.30, 1.00)	0.090

IgG avidity is expressed as molarity (M) at ID_{50}

Median (Q1, Q3)

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing.

Table 28. P-values for the comparison of serum IgG titres at each visit and the changes of IgG titres from baseline for Q-SRP and FM-SRP groups.

		p-value BAS	p-value Visit 2	p-value Visit 3	p-value Visit 4	p-value Visit 5	p-value Visit 6	p-value BAS-2	p-value BAS-3	p-value BAS-4	p-value BAS-5	p-value BAS-6
P.g.	Q-SRP	0.50	0.85	0.98	0.75	0.62	0.87	0.85	0.69	0.56	0.53	0.29
	FM-SRP											
A.a.	Q-SRP	1.00	0.74	0.40	0.89	0.98	0.68	0.28	0.24	1.00	0.82	0.38
	FM-SRP											
P.i.	Q-SRP	0.26	0.22	0.20	0.57	0.40	0.07	0.94	0.73	0.19	0.11	0.82
	FM-SRP											
T.d.	Q-SRP	0.62	0.59	0.43	0.60	0.89	1.00	0.77	0.88	0.01	0.10	0.26
	FM-SRP											
B.f.	Q-SRP	0.76	0.46	0.19	0.38	0.21	0.17	0.48	0.35	0.84	0.91	0.96
	FM-SRP											

P-values are given for differences between Q-SRP and FM-SRP groups

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*.

The comparison of the changes in IgG avidity (M at ID_{50}) between Q-SRP and FM-SRP groups over the six visits showed a significantly greater increase in IgG avidity to *T. denticola* for the Q-SRP group between BAS and visit two ($p<0.01$) (Table 29). When IgG avidity was compared between the two treatment groups at each visit with no reference to baseline, no significant differences were found for any organism (Table 29).

4.2 Discussion

4.2.1 Immunological responses after SRP

4.2.1.1 Serum IgG antibody titres

In general, decreases in serum IgG antibody titres to all putative pathogens were seen at six months. These results agree with data reported by other investigators (Tolo et al. 1982; Naito et al. 1985; Mouton et al. 1987; Aukhil et al. 1988; Murray et al. 1989; Horibe et al. 1995). However, significant variation exists among studies with respect to the duration of the treatment and the sampling intervals. In the current study, scaling and root planing was completed within six weeks for the Q-SRP group and within a day for the FM-SRP group. Both treatments resulted in decreased serum antibody titres, with the more marked reductions seen at six months. Most of the studies mentioned showed reductions in the antibody titres over a period of 12 months, but it has to be taken into account that the active phase of treatment in these studies lasted notably longer than that in the present study. However, other studies have demonstrated decreases in serum antibody levels in the immediate post-treatment period (Aukhil et al. 1988; Horibe et al. 1995).

Significant reductions in serum antibody levels against *P. gingivalis* and not for the other test bacteria have been demonstrated (Tolo et al. 1982; Naito et al. 1985). Horibe et al. (1995) showed significant reductions against *P. gingivalis* and *P. intermedia* from a large range of other putative periodontal pathogens. In contrast, the current study showed a significant reduction in the median antibody titres to the majority of the organisms tested and a concomitant decrease in the mean clinical parameters at six months.

Nevertheless, the present findings are inconsistent with those of Ebersole et al. (1985c), who showed that antibody titres increased two to four months post-scaling and returned

Table 29. P-values for the comparison of IgG avidity at each visit and the changes in IgG avidity from baseline for Q-SRP and FM-SRP groups.

		p-value BAS	p-value Visit 2	p-value Visit 3	p-value Visit 4	p-value Visit 5	p-value Visit 6	p-value BAS-2	p-value BAS-3	p-value BAS-4	p-value BAS-5	p-value BAS-6
P.g.	Q-SRP FM-SRP	0.46	0.48	1.00	0.94	0.71	0.91	0.65	0.60	0.28	0.25	0.51
A.a.	Q-SRP FM-SRP	0.31	0.14	0.21	0.90	0.13	0.37	0.32	0.42	0.62	0.87	0.31
P.i.	Q-SRP FM-SRP	0.60	0.74	0.41	0.50	0.19	0.74	0.27	0.85	0.85	0.71	0.33
T.d.	Q-SRP FM-SRP	0.11	0.42	0.78	0.17	0.45	0.12	<0.01	0.11	0.78	0.54	0.78
B.f.	Q-SRP FM-SRP	0.21	0.46	0.19	0.38	0.21	0.17	0.87	0.45	0.92	0.51	0.56

P-values are given for differences between Q-SRP and FM-SRP groups

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus.*

to pre-scaling levels eight to 12 months after treatment. In the current study, no increases in IgG titres to any of the organisms tested were detected post-scaling to support the inoculation effect of root planing on the host tissues as suggested by Ebersole et al. (1985c). However, since the participants in this study were followed over a period of approximately six months, no long-term comparisons can be made between the present findings and those of Ebersole et al. (1985c). Similarly, increases in serum IgG antibody titres to *A. actinomycetemcomitans* with decreases in pocket depths post-treatment have been demonstrated (Sjöström et al. 1994). The main difference between that study and the current study is that the former investigation examined a group with generalised aggressive periodontitis patients who required extensive and repeated treatment over a period of time and this may have boosted their humoral immune response. Mooney et al. (1995) showed elevated antibody titres to *A. actinomycetemcomitans* six weeks after the completion of therapy. Their results, in addition to those of Chen et al. (1991), showed increased antibody titres to *A. actinomycetemcomitans* and *P. gingivalis*, respectively, for the seronegative patients after treatment. However, the current study did not examine the immune responses based on the initial serostatus of the patients.

In conclusion, data reported here confirm those by Mouton et al. (1987) who showed a progressive reduction in antibody titres to *P. gingivalis* five to seven months post-treatment, implying that root planing does not cause active immunisation with *P. gingivalis*. Periodontal therapy appears to decrease the antigenic load post-treatment resulting in lower levels of antibodies.

4.2.1.2 GCF IgG antibody titres

In general, the present study revealed low values of GCF titres with a wide site-to-site variability among sites of similar periodontal status. This is in accordance with results from other studies (Ebersole et al. 1984; Tew et al. 1985). Antibody titres were much lower in GCF than in serum and this finding confirms previous results (Ebersole et al. 1985a; Baranowska et al. 1989). A recent study demonstrated little change in the local and systemic antibody levels after treatment, emphasising the wide variation in the subjects' response to treatment (Darby et al. 2001).

Results from the present study showed a significant reduction in GCF volume after therapy. In addition, at six months there was a significant decrease in GCF antibody titres to *A. actinomycetemcomitans* and *T. denticola* for the Q-SRP group and to *P. intermedia* and *T. denticola* for the FM-SRP group. It should be noted that serum IgG titres to these organisms decreased after treatment, albeit not significantly for all species and that the presence of these pathogens in subgingival plaque was markedly reduced post-therapy (these findings will be discussed in Chapter five). These data indicate that a reduction in the bacterial burden coincides with a reduction in the local and systemic antibody levels.

Of great interest was the finding that GCF volume was significantly negatively correlated with GCF antibody titres to *P. gingivalis* at six months, indicating that although GCF volume decreased after treatment, there were still high antibody titres to this organism in the GCF. It must be stressed that although GCF antibody titres decreased for some of the bacteria post-therapy, there was a tendency for antibody concentration in GCF to increase. This was due to the fact that GCF volume significantly decreased following treatment and this compensated for the reduction in GCF antibody titres. One possible reason for this could be that the organisms still stimulated and mounted a local immune response, even though their presence in the sampled pockets was not detected. This may reflect the potential of a pathogen (i.e. *P. gingivalis*) to invade the gingival tissues (Dzink et al. 1989; Duncan et al. 1993), from where it generates a local immune response with the production of specific antibodies. Alternatively, organisms at other oral sites and niches that were not sampled may have induced the elevated antibody levels. Another possibility is that the microbiota is in a state of dynamic flux and that a single sampling does not accurately reflect the flora.

It has been shown that there is local antibody production in diseased gingival tissues and that this response is specific (Lally et al. 1980). Therefore, elevated antibody titres in GCF may reflect changes in disease activity at specific sites that are not detectable by conventional clinical measurements, or by changes in the systemic immune responses. Conversely, the tendency for the concentration of GCF antibodies to increase post-treatment, despite the decrease in homologous serum antibody titres could be explained by the fact that the organisms that were formerly present in large numbers in the pockets pre-treatment decreased dramatically after scaling, resulting in less antibody

consumption locally and therefore excess of free antibodies in the gingival crevice. It has been demonstrated that GCF IgG levels were lower in periodontitis sites than in gingivitis sites in the same patient, implying that increased consumption of locally produced antibody in periodontitis sites may be responsible for this difference (Mooney and Kinane, 1997). In addition, Smith et al. (1985) showed lower antibody levels in the GCF than in the gingival homogenates, possibly due to consumption of local antibodies by organisms present in the crevice.

4.2.1.3 IgG antibody avidity

During infection, high levels of antibodies are produced that are of low specificity or avidity (O'Dell and Ebersole, 1995). Numerous studies compared the antibody levels and avidity between subjects with periodontitis and healthy controls. Antibodies of higher avidity in the healthy individuals than the diseased were demonstrated suggesting that antibodies of high avidity are protective against disease (Chen et al. 1991; Whitney et al. 1992; Saito et al. 1993). However, conflicting data exist that show no significant differences in IgG antibody avidities between periodontitis patients and healthy subjects (Mooney et al. 1993; O'Dell and Ebersole, 1995). Another study has demonstrated increased antibody avidity of the IgG class in periodontitis patients compared to healthy controls possibly as a result of repeated antigenic challenge with the same pathogens (Lopatin et al. 1991).

Successful treatment results in the elimination of the aetiological agents and maturation of the immune system to produce antibodies of high avidity (Chen et al. 1991). Mooney et al. (1995) found an increase in IgG avidity to *P. gingivalis* ($p=0.05$) six weeks after hygiene phase therapy. In the present study, IgG avidity showed a trend towards increasing post-treatment for the majority of the organisms tested, but generally this finding did not reach statistical significance for most of the test bacteria. It has been confirmed in other studies that antibody avidity is independent of the antibody levels (O'Dell and Ebersole, 1995). Data reported here show that treatment resulted in lower levels of antibodies to all the bacteria tested, but avidity remained the same for the majority of the organisms tested. Another study from our laboratory showed that despite the clinical improvement seen post-therapy, there were no significant post-treatment effects on the humoral immune response other than a reduction in the avidity of antibodies to *P. gingivalis* and *P. intermedia* (Darby et al. 2001). This finding may

indicate a failure of the host response to produce adequate levels of biologically functional antibodies after treatment. It is possible that this poor host response makes patients susceptible to future disease progression.

The present findings imply that antibody avidity may be a very dynamic process, with fluctuations that are difficult to detect with conventional laboratory techniques, or that a period of time longer than the monitoring period of this study (six months) is required to document the maturation of the immune system. Lopatin et al. (1991) demonstrated that human IgG antibodies were of extremely low avidity compared to the avidity levels in rabbits immunised with *P. gingivalis*. These results suggest that the minimal immune maturation seen in human IgG antibodies to *P. gingivalis* could be a result of an *in utero* tolerization to bacterial antigens or of chronic exposure to oral bacteria after birth. Bearing this in mind, although the reduction in the antibody levels noted in the present study possibly reflects the reduction in the antigenic load, the immune system may still be challenged with antigens that consume the higher avidity antibodies and remove them from the circulation. However this seems unlikely, as it has been demonstrated that healthy individuals had IgG antibodies of lower avidity than did periodontitis patients, who are expected to have a greater potential for antibody consumption than the former subjects (Lopatin et al. 1991). Therefore, it is possible that due to repeated antigenic exposure during the chronic process of periodontal disease the antibody avidity of patients in the current study was elevated pre-treatment, and it reached a threshold that could not be surpassed after treatment. Another hypothesis is that the chronic exposure to periodontopathogens may inhibit the maturation of the immune system. However, these pathogenetic mechanisms still need clarification and more evidence is required to support these concepts.

4.2.2 Comparison of immunological parameters in Q-SRP and FM-SRP groups

The present study showed that there were no significant differences in IgG avidity between the two treatments. Although antibody titres were similar for both groups at baseline, R1 and R2, a significantly greater reduction in the antibody levels to *P. intermedia* and *T. denticola* was seen between BAS and R1 for the FM-SRP group than the Q-SRP group. When the changes in the serum antibody response with treatment were considered within each group, significant reductions in the antibody levels to *P. intermedia* and *T. denticola* were seen between BAS and R1 for the FM-SRP group, but

this was not seen against any of the tested organisms for the Q-SRP group. These findings imply that FM-SRP seems to have a stronger short-term effect on the systemic antibody response compared to the classical therapy of quadrant root planing at two-weekly intervals. However, the clinical significance of this finding is difficult to assess.

The analysis of the local antibody responses revealed that the FM-SRP group had higher GCF antibody titres to *T. denticola* at R1 and to *P. intermedia* at R2 than did the Q-SRP group. Given the fact that selected sites of the FM-SRP patients showed higher PD indices at R1 than did the Q-SRP patients, this finding may reflect that there is an ongoing healing process at the former sites with higher levels of specific local antibodies.

4.2.3 Dynamics of antibody response after Q-SRP and FM-SRP

There is evidence of a short-term effect of treatment on the antibody response. Horibe et al. (1995) found a significant reduction in the antibody levels at least two months after the completion of treatment. Aukhil et al. (1988) showed significantly lower antibody levels by the end of the hygiene phase of treatment, i.e. approximately two months after baseline. The current study is the first that has attempted to determine the dynamics of the antibody response during the active phase of treatment, i.e. at two-weekly intervals over a period of three months. Data reported here failed to show that each session of root planing resulted in increased antibody levels due to an inoculation effect of bacteria in the host tissues as suggested by Ebersole et al (1985c). Each session of root planing seemed to result in antibodies of either similar or significantly lower levels compared to baseline values. These results agree with those of Mouton et al. (1987), who demonstrated a gradual reduction in antibody levels five to seven months after treatment with no peak antibody levels seen in the immediate post-scaling period. In addition, in the short-term no cyclic pattern of antibody levels was seen to justify the series of exacerbation and remission of disease activity as has been suggested previously by Socransky et al. (1984).

Antibody avidity appeared to increase during the active phase of treatment for both treatment groups and this reached statistical significance even after the first scaling visits, reflecting the production of high avidity antibodies as a result of antigenic elimination and / or immune maturation. However, of great interest was the observation

that antibody avidity significantly increased in the immediate post-scaling period rather than at later stages (R1 and R2). This finding, if upheld by further studies, would contradict the hypothesis that the baseline values of antibody avidity in periodontitis patients exceed a threshold beyond which no significant increases can be noted. However, it is difficult to interpret these results given the variations within and between individuals and over time. It should be borne in mind that this study did not compare the antibody response between healthy subjects and treated periodontitis patients. Therefore, any fluctuations in the antibody avidity that may be caused by factors not related to the periodontal therapy (i.e. Hawthorne effect or other environmental influences except for cigarette smoking) were not disclosed.

No significant differences in serum antibody levels and avidity were seen between the two treatment groups at each visit during the active phase of treatment (baseline and three additional visits). However, comparison of changes in serum antibody levels and avidity with treatment between Q-SRP and FM-SRP groups revealed a significantly greater reduction in serum antibody levels to *T. denticola* for the FM-SRP group between BAS and visit four, and a significantly lower increase in antibody avidity to *T. denticola* for the FM-SRP group between BAS and visit two. It must be stressed however, that visit four corresponded to R1 for the FM-SRP group and to the last session of quadrant root planing for the Q-SRP group. This means that the assessment of the systemic immunological parameters at this time point was made before treatment was completed in the Q-SRP group. These differences in the changes of antibody levels and avidity between the two treatment groups would appear to be of doubtful clinical significance.

The present findings do not confirm the hypothesis that the beneficial effects of FM-SRP with and without the use of chlorhexidine are due to an acute immunological reaction caused by the inoculation of bacteria into the host tissues (Quirynen et al. 2000). These authors speculated that this reaction occurs one to two days after the FM-SRP treatment and is reflected by an increase in the patients' body temperature. However, they did not examine the dynamics of the antibody response after FM-SRP or whether this treatment induced bacteraemia. Results from the current study did not reveal elevated antibody levels during the course of treatment to support the hypothesis that one-day FM-SRP caused bacteraemia. Q-SRP also has the potential to stimulate an

elevated immune response, by repeatedly inoculating bacteria into the tissues over consecutive sessions of root planing. However, even though these immunological inoculations may occur, the present study was unable to confirm this for a variety of reasons, which may include inter- and intra-subject variability in response to therapy, extent and severity of disease, timing effects and differences in oral hygiene standards. Other enviromental and host response factors, which can not be estimated easily may also account for that.

CHAPTER 5

EFFECT OF SCALING AND ROOT PLANING ON MICROBIOLOGICAL PARAMETERS

5. Effect of scaling and root planing on microbiological parameters

5.1 Results

5.1.1 PCR prevalence of putative periodontal pathogens

PCR analysis revealed that at a site-specific level (n=159) the baseline detection frequencies of the specific organisms were: 32.7% for *P. gingivalis*; 22.6% for *A. actinomycetemcomitans*; 36.4% for *P. intermedia*; 44.7% for *T. denticola*; and 80.5% for *B. forsythus*. On a subject-basis (n=42), (i.e. whether a subject harboured the organism at any site) the baseline prevalence of the bacteria was: 38.0% for *P. gingivalis*; 33.3% for *A. actinomycetemcomitans*; 59.5% for *P. intermedia*; 76.2% for *T. denticola*; and 92.9% for *B. forsythus* (data not shown). Figures 9-13 depict the results of the PCR assay to detect the five putative periodontal pathogens in subgingival plaque samples.

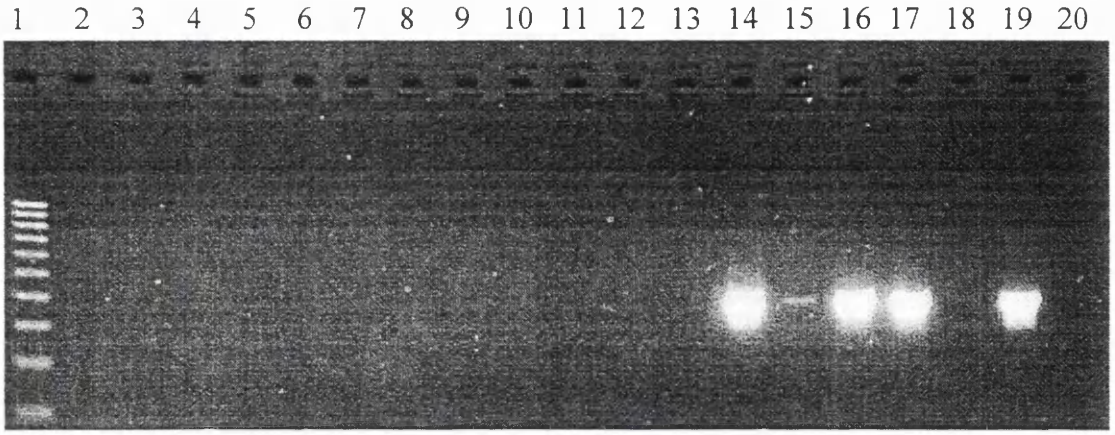
5.1.2 Bacterial combinations

Figure 14, shows the bacterial combinations in selected sites at baseline. A high percentage of sites were positive for the combination of *P. gingivalis* and *B. forsythus* (30%), *P. intermedia* and *B. forsythus* (36%) and *T. denticola* and *B. forsythus* (42%). 9% of the sites (n=159) were negative for any of the five putative pathogens or their combinations.

5.1.3 Subgingival microflora before and after Q-SRP

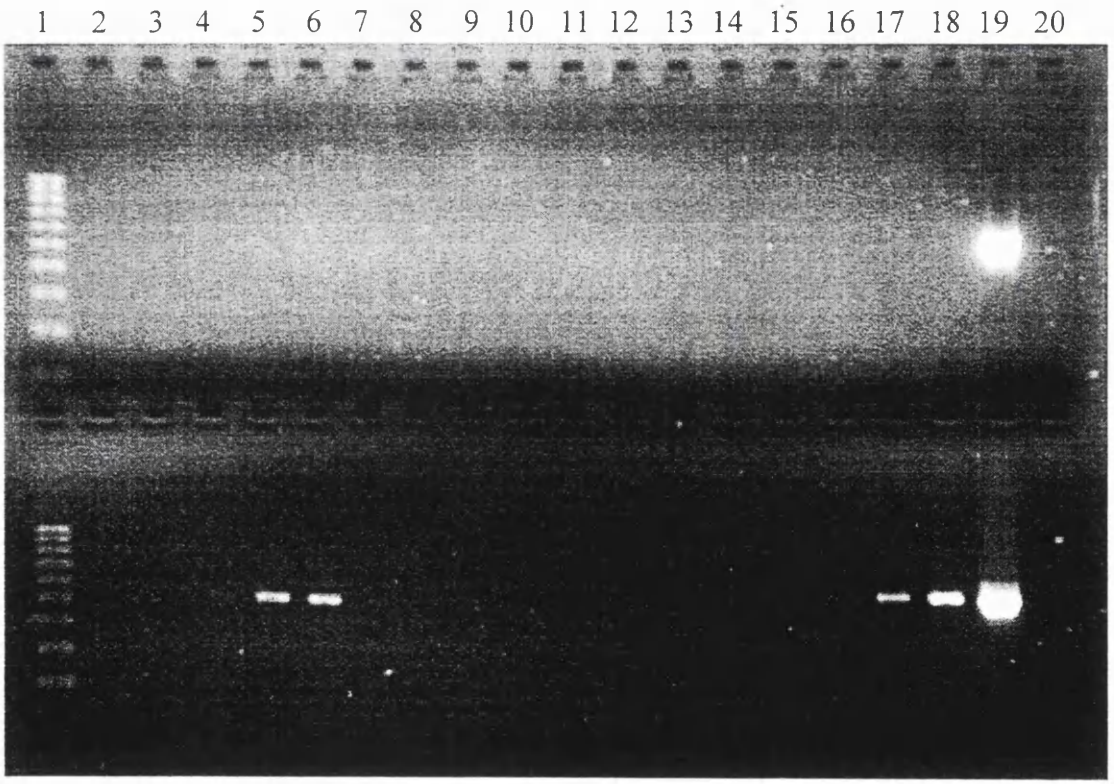
Microbiological data from 80 sites were analysed for Q-SRP. There was a marked reduction in the presence of all five putative periodontal pathogens at selected sites following Q-SRP. This altered microbiota was maintained at R2 (Table 30). The percentage of sites positive for *P. gingivalis* was significantly reduced at R1 and R2 ($p<0.001$). Breakdown refers to the sites that were negative for a pathogen at baseline and became positive for the same organism post-treatment. One site that was negative for *P. gingivalis* at baseline turned positive at R1 but none at R2. The detection frequency of *A. actinomycetemcomitans* at selected sites was significantly reduced at R1 and R2 ($p<0.005$). Two sites initially free for *A. actinomycetemcomitans* became positive for this organism between BAS and R1 and between BAS and R2. The detection frequencies of *P. intermedia*, *T. denticola* and *B. forsythus* significantly decreased at R1 ($p<0.001$) and R2 ($p<0.001$). Four sites that were formerly negative for *P. intermedia* became positive at R1 and two sites at R2 from BAS. One site that was

Figure 9. *P. gingivalis* PCR product analysis.



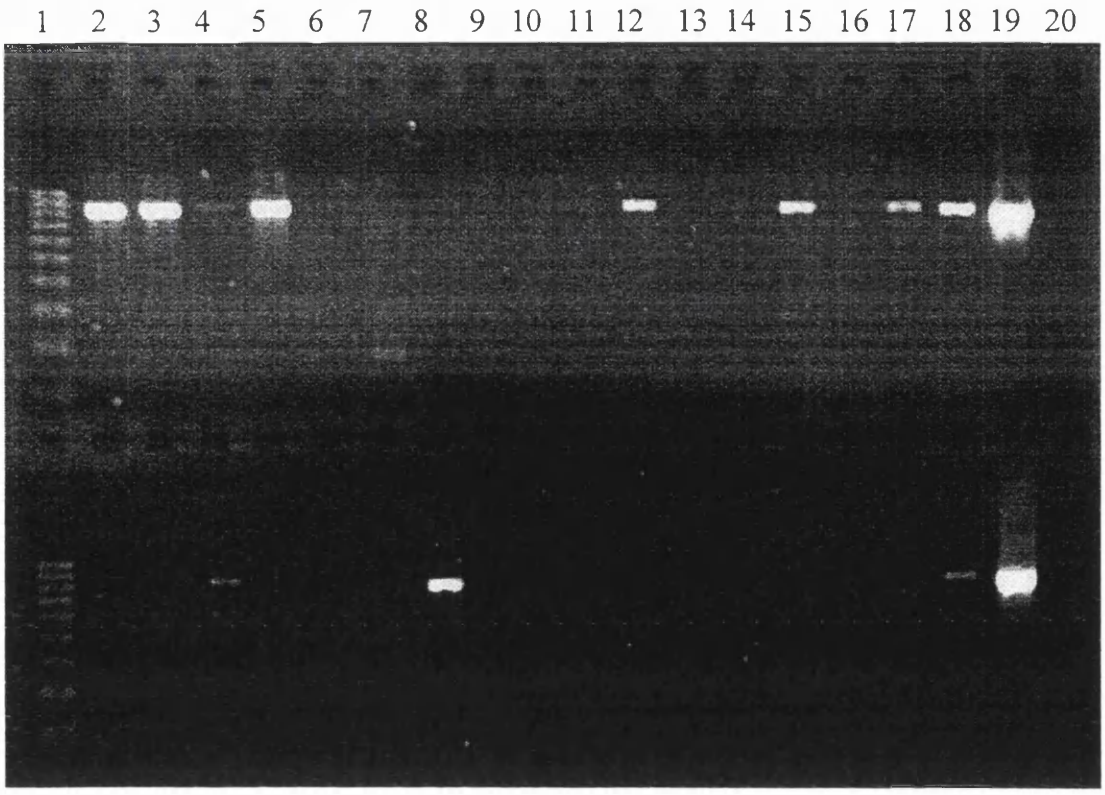
Lane 1: 100-bp DNA ladder; lanes 2-18: plaque samples; lane 19: positive control; lane 20: negative control.

Figure 10. *A. actinomycetemcomitans* PCR product analysis.



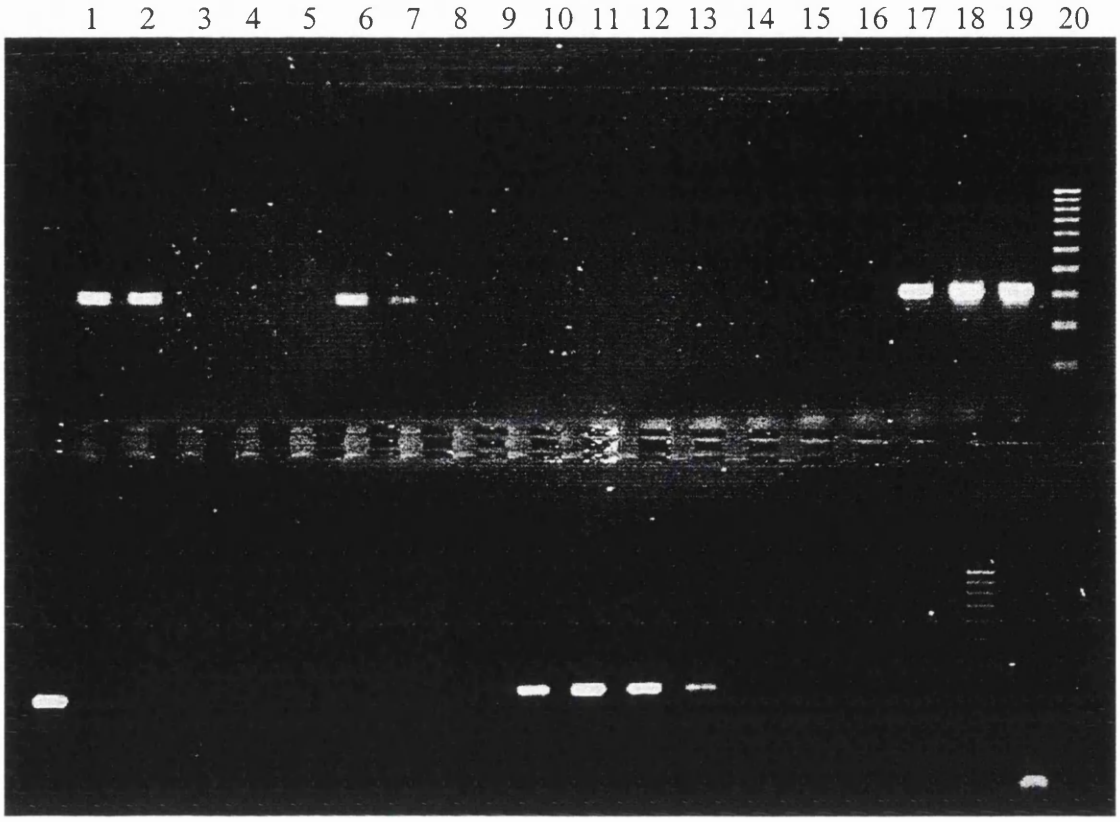
Upper and lower panels: lane 1: 100-bp DNA ladder; lanes 2-18: plaque samples; lane 19: positive control; lane 20: negative control.

Figure 11. *P.intermedia* PCR product analysis.



Upper and lower panels: lane 1: 100-bp DNA ladder; lanes 2-18: plaque samples; lane 19: positive control; lane 20: negative control.

Figure 12. *T. denticola* PCR product analysis.

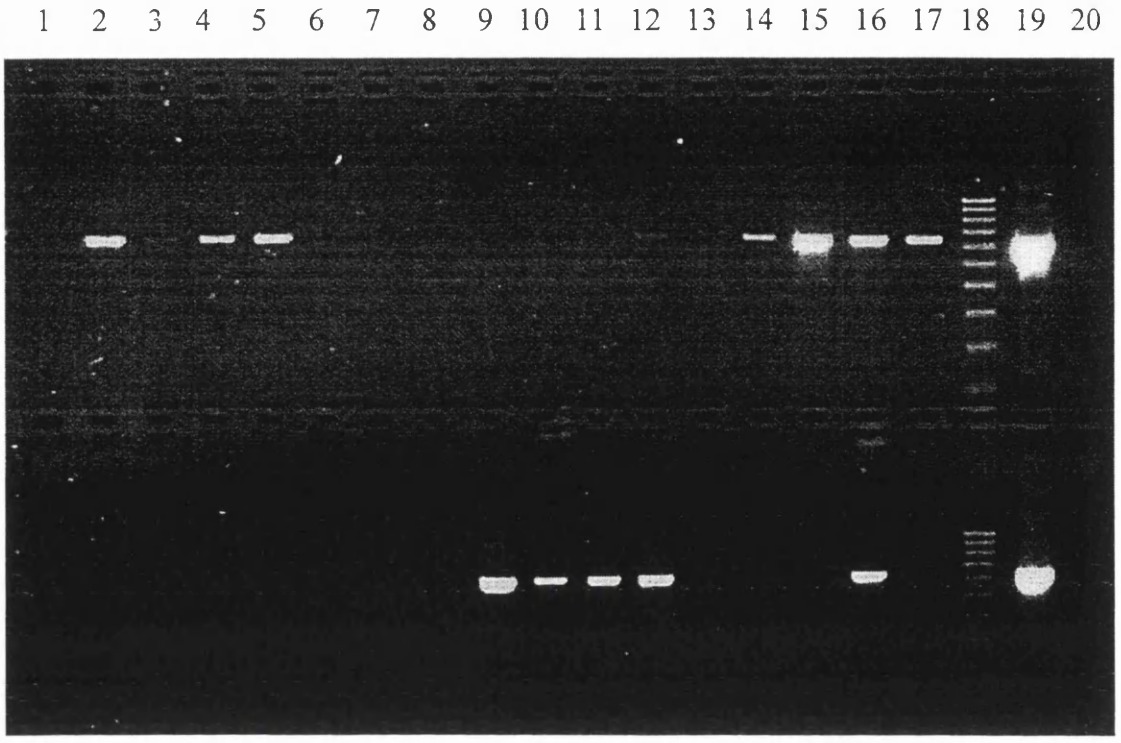


Upper panel: lanes 1-19: plaque samples; lane 20: 100-bp DNA ladder;

Lower panel: lanes 1-17: plaque samples; lane 18: 100-bp DNA ladder; lane 19: positive control; lane 20: negative control.

The positive control used was a small fragment of the *T. denticola* 16S rRNA gene which was 79 bp in length. This comprised the first 52 bp of the full-length PCR product and the downstream primer sequence.

Figure 13. *B. forsythus* PCR product analysis.



Upper and lower panels: lanes 1-17: plaque samples; lane 18: 100-bp DNA ladder; lane 19: positive control; lane 20: negative control.

Figure 14. Bacterial combinations.

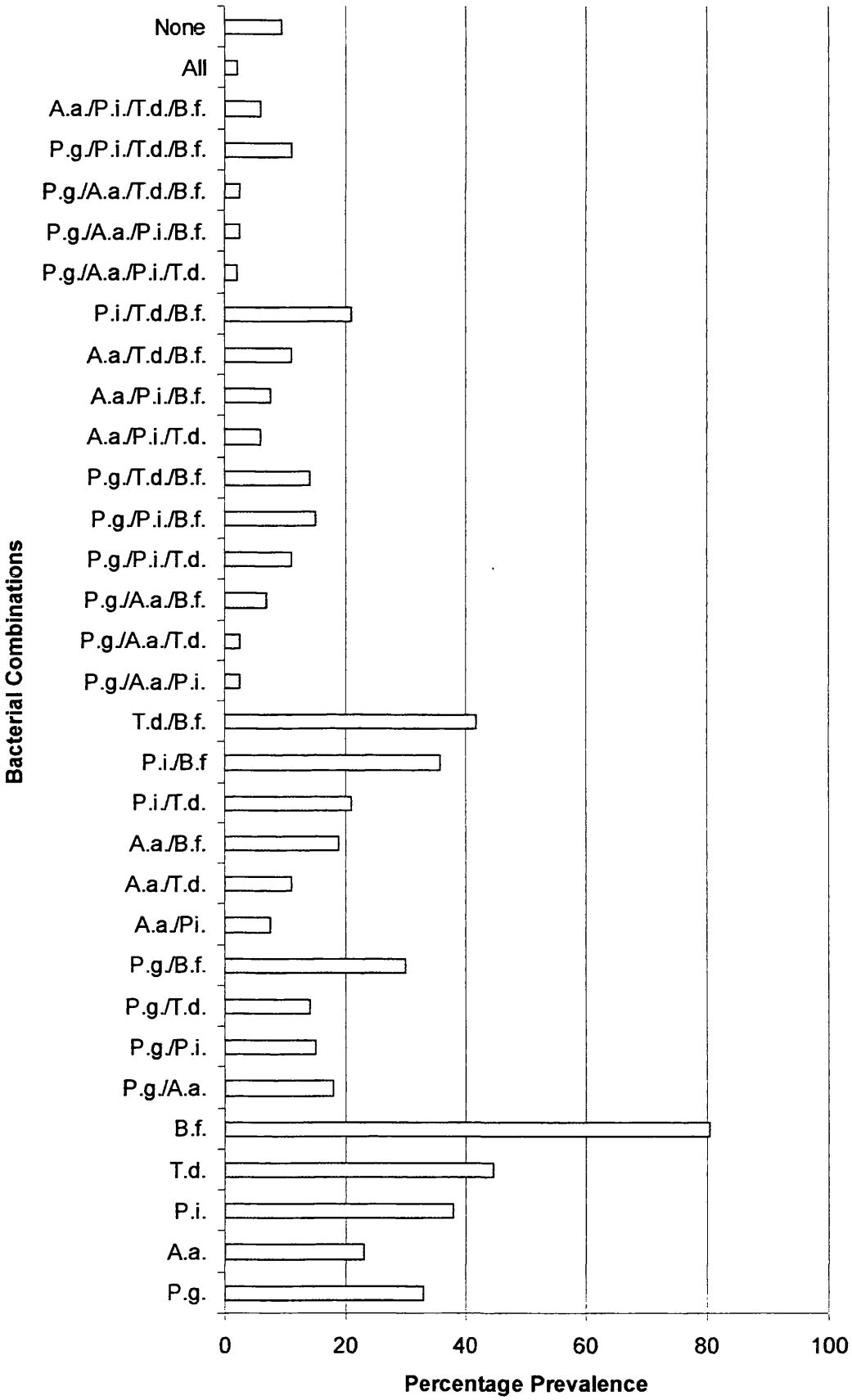


Table 30. Percentage of sites positive for the five putative periodontal pathogens before and after Q-SRP.

N=80	BAS	R1	BAS-R1		p– value	R2	BAS-R2		p– value
			Breakdown	Change	(BAS-R1)		Breakdown	Change	(BAS-R2)
P.g.	35.00	1.25	1.25	33.75	<0.001	0.00	0.00	35.00	<0.001
A.a.	21.25	5.00	2.50	16.25	0.002	6.25	2.50	15.0	0.004
P.i.	40.00	11.25	5.00	28.75	<0.001	11.25	2.50	28.75	<0.001
T.d.	42.50	3.75	1.25	38.75	<0.001	3.75	0.00	38.75	<0.001
B.f.	77.50	8.75	1.25	68.75	<0.001	11.25	1.25	66.25	<0.001

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

Breakdown: % of negative sites at baseline that turned positive post-treatment.

negative for *T. denticola* at BAS became positive for this pathogen at R1, and no sites at R2 from BAS. *B. forsythus* colonised one site that was formerly negative for this species at R1 and one site at R2 from BAS.

When the analysis was made on a patient basis (n=20), a marked reduction in the percentage of patients that were positive for the five periodontal pathogens post-treatment was also demonstrated (Table 31). The percentage of subjects that harboured *P. gingivalis* significantly decreased at R1 ($p<0.05$) and R2 ($p<0.01$). One patient who was initially negative for *P. gingivalis* became positive between BAS and R1. In addition, *T. denticola* was significantly reduced at R1 ($p<0.05$) and R2 ($p<0.005$) and this was the case with *B. forsythus* ($p<0.005$). One subject formerly negative for *B. forsythus* turned positive for this organism after treatment and one patient became positive for *T. denticola* at R1 from BAS. No statistically significant decreases in the prevalence of *A. actinomycetemcomitans* and *P. intermedia* were noted following treatment. One patient who was negative for *A. actinomycetemcomitans* at BAS, turned positive for this pathogen at R1 and one at R2 from BAS. Two patients were colonised with *P. intermedia* between BAS and R1 and two between BAS and R2.

5.1.4 Subgingival microflora before and after FM-SRP

Due to technical difficulties, three patients in the FM-SRP group had incomplete sets of plaque samples at baseline, although they finished the study. Therefore, the analysis of the microbiological data for this treatment group was performed on 79 subgingival plaque samples at baseline and R1 and on 76 at R2.

Table 32 shows the prevalence of the five periodontal pathogens before and after FM-SRP on a site-based analysis. The percentage of sites positive for *P. gingivalis* was significantly reduced at R1 and R2 ($p<0.001$). No colonisation of *P. gingivalis* at initially negative sites occurred post-treatment. Similarly, the detection frequency of *A. actinomycetemcomitans* significantly decreased at R1 ($p<0.05$) and R2 ($p<0.001$). Three sites negative for *A. actinomycetemcomitans* at baseline were colonised by this microorganism between BAS and R1 and BAS and R2. *P. intermedia*, *T. denticola* and *B. forsythus* showed significant reductions in their detection frequencies at the selected sites at R1 ($p<0.001$) and R2 ($p<0.001$). Two sites initially negative for *P. intermedia* were colonised by this species between BAS and R2. At R2, *T. denticola* colonised one

Table 32. Percentage of sites positive for the five putative periodontal pathogens before and after FM-SRP.

N=79	BAS	R1	BAS-R1		p– value	R2	BAS-R2		p– value
			Breakdown	Change	(BAS-R1)		Breakdown	Change	(BAS-R2)
P.g.	30.4	2.3	0.0	27.8	<0.001	1.3	0.0	29.9	<0.001
A.a.	24.0	10.2	4.0	12.7	0.021	1.3	0.0	26.9	<0.001
P.i.	36.7	1.1	0.0	35.5	<0.001	7.9	3.0	28.4	<0.001
T.d.	46.8	0.0	0.0	46.8	<0.001	4.0	1.5	44.8	<0.001
B.f.	83.5	4.5	0.0	78.5	<0.001	7.9	1.5	73.1	<0.001

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

Breakdown: % of negative sites at baseline that turned positive post-treatment.

site formerly free of this pathogen and one site that was initially negative for *B. forsythus* turned positive between BAS and R2.

The patient-based analysis demonstrated that there was a statistically significant reduction in the percentage of patients positive for all putative pathogens post-treatment (Table 33). The percentage of patients positive for *P. gingivalis* significantly decreased at R1 ($p<0.05$) and R2 ($p<0.05$) and the percentage of patients that harboured *A. actinomycetemcomitans* significantly decreased at R2 only ($p<0.05$). Significantly fewer patients possessed *P. intermedia* at R1 ($p=0.001$) and R2 ($p<0.05$). Similarly, the detection frequencies of *T. denticola* and *B. forsythus* were significantly reduced at R1 ($p<0.001$) and R2 ($p=0.001$). Post-treatment, *B. forsythus* colonised one subject that was free of this species at baseline, while this was not seen for the other bacteria.

5.1.5 Comparison of microbiological responses between Q-SRP and FM-SRP groups

At baseline, there were no significant differences for any of the test bacteria between Q-SRP and FM-SRP on a site-based analysis (Table 34). A comparison between the two treatments at each visit showed a significantly higher percentage of sites positive for *P. intermedia* at R1 for the Q-SRP group than the FM-SRP group ($p<0.01$). However, when the changes with treatment were compared, no significant differences were seen between the two groups. These seemed to be similar for both treatments.

Table 35, shows the patient-based analysis of the PCR data. At baseline, a significantly lower percentage of Q-SRP patients (55.0%) was positive for *T. denticola* while 95.5% of patients in FM-SRP group harboured this pathogen ($p<0.01$). At R1, *P. intermedia* was more prevalent in Q-SRP patients than FM-SRP patients ($p<0.05$). At R2, *P. gingivalis* was not detected in the Q-SRP group, while 5.2% of the FM-SRP patients were positive for this pathogen. *T. denticola* was present in 15% of the Q-SRP patients at R1 but was not detected in the FM-SRP group. However, these differences between the groups were not statistically significant. When the changes with treatment were analysed, a significantly greater reduction in the proportion of patients positive for *T. denticola* was seen in the FM-SRP group at both R1 ($p<0.01$) and R2 ($p=0.01$), but any conclusion from this comparison between the two treatment groups is unclear given their significant baseline differences.

Table 33. Percentage of patients positive for the five putative periodontal pathogens before and after FM-SRP.

N=22	BAS	R1	BAS-R1		p– value	R2	BAS-R2		p– value
			Breakdown	Change	(BAS-R1)		Breakdown	Change	(BAS-R2)
P.g.	36.4	4.5	0.0	31.8	0.016	5.2	0.0	31.6	0.031
A.a.	31.8	13.6	0.0	18.2	0.125	5.3	0.0	31.6	0.031
P.i.	54.5	4.5	0.0	50.0	0.001	26.3	0.0	31.6	0.031
T.d.	95.5	0.0	0.0	95.5	<0.001	10.5	0.0	84.2	<0.001
B.f.	95.5	13.6	0.0	81.8	<0.001	26.3	5.0	68.5	0.001

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

Breakdown: % of negative patients at baseline that turned positive post-treatment

Table 34. Percentage of sites positive for the five putative periodontal pathogens before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=80 $N_{FM\text{-}SRP}$=79		Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g.	Q-SRP	35.00	0.530	1.25	0.990	0.00	0.490	33.75	0.330	35.00	0.510
	FM-SRP	30.40		2.30		1.30		27.80		30.00	
A.a.	Q-SRP	21.25	0.670	5.00	0.200	6.25	0.210	16.25	0.700	15.00	0.170
	FM-SRP	24.00		10.20		1.30		12.70		26.90	
P.i.	Q-SRP	40.00	0.670	11.25	0.007	11.25	0.480	28.75	0.820	28.75	0.990
	FM-SRP	36.70		1.10		7.90		35.40		28.40	
T.d.	Q-SRP	42.50	0.580	3.75	0.100	3.75	0.990	38.75	0.390	38.75	0.360
	FM-SRP	46.80		0.00		4.00		46.80		44.80	
B.f.	Q-SRP	77.50	0.340	8.75	0.270	11.25	0.480	68.75	0.220	66.25	0.340
	FM-SRP	83.50		4.50		7.90		78.50		73.10	

P-values are given for differences between Q-SRP and FM-SRP groups

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 35. Percentage of patients positive for the five putative periodontal pathogens before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=20 $N_{FM\text{-}SRP}$=22		Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g.	Q-SRP	40.0	0.810	5.0	1.000	0.0	0.490	35.0	0.580	40.0	0.580
	FM-SRP	36.4		4.5		5.2		31.8		31.6	
A.a.	Q-SRP	35.0	0.830	15.0	1.000	15.0	0.600	20.0	0.710	20.0	0.650
	FM-SRP	31.8		13.6		5.3		18.2		31.6	
P.i.	Q-SRP	65.0	0.490	35.0	0.018	35.0	0.560	30.0	0.520	30.0	0.580
	FM-SRP	54.5		4.5		26.3		50.0		31.6	
T.d.	Q-SRP	55.0	0.003	15.0	0.100	10.0	1.000	40.0	<0.001	45.0	0.010
	FM-SRP	95.5		0.0		10.5		95.5		84.2	
B.f.	Q-SRP	90.0	0.600	35.0	0.150	35.0	0.560	55.0	0.118	55.0	0.370
	FM-SRP	95.5		13.6		26.3		81.8		68.4	

P-values are given for differences between Q-SRP and FM-SRP groups

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

5.1.6 Investigation of possible re-colonisation of treated sites by pathogens from the remaining untreated periodontal pockets

An attempt was made to test whether the scaled sites were re-colonised by pathogens from the remaining untreated periodontal pockets or whether root planing the first quadrant gave a host-induced benefit to the other quadrants. Therefore, the clinical and microbiological parameters of sites in quadrant one (1Q) were compared with those of sites in quadrant four (4Q) following Q-SRP therapy. It should be stressed that 1Q was always root planed first (upper right quadrant), while 4Q (lower right quadrant) was treated last.

The comparison of the clinical indices between sites in 1Q and 4Q demonstrated an unfortunate chance bias in that 1Q on average had significantly deeper pockets at baseline than 4Q, 6.8 mm versus 5.8 mm ($p<0.05$) (Table 36). In addition, sites in 1Q remained deeper at R1 and R2 in comparison to 4Q but this was not statistically significant and is an unfair comparison given the significant difference in the baseline values. No significant differences in PD reduction were noted between the two quadrants after treatment. There was no significant difference in RAL between 1Q and 4Q before and after treatment. In addition, no significant difference in gain of RAL was found between 1Q and 4Q at R1 and R2 from BAS. BOP was similar for 1Q and 4Q at BAS and at R1 and R2. Despite the greater BOP reduction between BAS and R2 for 4Q, this was not statistically significant. Sites in both quadrants showed a continuous clinical improvement three and six months post-therapy.

No significant differences in the prevalence of putative periodontal pathogens were found between sites in 1Q and 4Q at baseline and following Q-SRP (Table 37). In addition, the changes in the prevalence of bacteria at R1 and R2 from BAS were not significantly different between 1Q and 4Q.

The changes in the prevalence of pathogens in 1Q following therapy showed significant reductions for *P. gingivalis* between BAS and R2 ($p<0.05$), for *T. denticola* at R1 ($p<0.05$) and R2 ($p<0.01$) from BAS, and for *B. forsythus* at R1 ($p=0.001$) and R2 ($p<0.001$) from BAS. From this Table it is clear that, the percentage of sites in 1Q positive for the test pathogens was maintained at low levels at R1 and R2.

Table 36. Comparison of the clinical indices between sites in quadrant one and quadrant four before and after Q-SRP.

1Q=20 4Q=20		Baseline	p-value[2]	R1	p-value[2]	R2	p-value[2]	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
PD(mm)	1Q	6.8 ± 1.3	0.015	3.8 ± 1.1	0.180	3.5 ± 0.9	0.080	3.0 ± 1.2	<0.001	0.270	3.3 ± 1.4	<0.001	0.260
	4Q	5.8 ± 1.2		3.3 ± 1.3		3.1 ± 0.8		2.5 ± 1.5	<0.001		2.7 ± 1.5	<0.001	
RAL(mm)	1Q	14.9 ± 2.3	0.150	13.8 ± 2.4	0.450	13.6 ± 2.5	0.260	1.0 ± 0.9	<0.001	0.120	1.3 ± 0.8	<0.001	0.370
	4Q	13.9 ± 2.0		13.3 ± 2.1		12.8 ± 1.8		0.6 ± 1.0	0.013		1.0 ± 0.8	<0.001	
BOP*	1Q	70.0	0.240	15.0	1.000	20.0	0.670	55.0	0.003	0.510	50.0	0.013	0.080
	4Q	90.0		20.0		10.0		70.0	<0.001		80.0	<0.001	

Mean ± sd

* % of sites

PD = pocket depth; RAL = relative attachment level; BOP = bleeding on probing;

Q-SRP = quadrant scaling and root planing; 1Q: quadrant 1; 4Q: quadrant 4

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

[1]P-value is given for changes before/after Q-SRP for sites in 1Q and 4Q

[2]P-value is given for differences between 1Q and 4Q.

Table 37. Percentage of sites positive for the five putative periodontal pathogens in quadrant one and quadrant four before and after Q-SRP.

1Q=20 4Q=20		Baseline	p-value[2]	R1	p-value[2]	R2	p-value[2]	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
P.g.	Site 1Q	35	1.000	5	1.000	0		30	0.070	1.000	35	0.016	1.000
	Site 4Q	35		0		0		35	0.016		35	0.016	
A.a	Site 1Q	25	0.700	10	1.000	5	1.000	15	0.375	1.000	20	0.125	0.660
	Site 4Q	15		5		10		10	0.625		5	1.000	
P.i.	Site 1Q	40	0.500	20	0.700	10	0.700	20	0.290	0.500	30	0.070	0.740
	Site 4Q	50		10		20		40	0.008		30	0.031	
T.d.	Site 1Q	45	0.500	5	1.000	5	1.000	40	0.021	0.300	40	0.008	0.310
	Site 4Q	35		5		10		30	0.031		25	0.063	
B.f.	Site 1Q	85	1.000	15	1.000	10	0.400	70	0.001	0.500	75	<0.001	0.090
	Site 4Q	80		15		25		65	<0.001		55	0.001	

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing; 1Q: quadrant 1; 4Q: quadrant 4

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

[1]P-value is given for changes in 1Q and 4Q before/after Q-SRP

[2]P-value is given for differences between 1Q and 4Q.

5.1.7 Effect of the presence and absence of putative periodontal pathogens on clinical and immunological parameters

A comparison of site-specific clinical indices between sites that were positive and sites that were negative for the test bacteria found no significant differences in PD, RAL and BOP at baseline (Table 38). Nevertheless, sites that were positive for *B. forsythus* and *A. actinomycetemcomitans* had a higher frequency of suppuration. 67% of sites that harboured *B. forsythus* suppurated while this was the case for only 42% of the sites that were free of this organism ($p<0.01$). Suppuration was present in 78% of sites that were positive for *A. actinomycetemcomitans* versus 58% of sites that were negative for this pathogen ($p<0.05$).

The comparison of the full-mouth clinical indices of patients that were positive and negative for a pathogen yielded significant differences at baseline (Table 39). Patients that harboured *A. actinomycetemcomitans* had significantly lower PD, AL and No. of sites $\geq$ 5mm than those who were free of this pathogen. PD was 4.1 mm for positive patients versus 4.5 mm for negative patients ($p<0.05$), AL was 4.5 mm versus 5.3 mm ($p<0.005$), and the No. of sites $\geq$ 5mm was 55 versus 74 ($p<0.005$). On the contrary, patients positive for *T. denticola* had higher AL (5.2 mm) than negative patients (4.5 mm) ($p<0.05$) and those who were positive for *B. forsythus* had deeper pockets, (4.4 mm versus 3.6 mm) ($p=0.01$), and more pockets with PD $\geq$ 5mm than negative patients (70 versus 44) ($p<0.005$).

GCF IgG titres (EU / 30 sec) were compared between sites that were positive and negative for a pathogen at baseline (Table 40). Antibody levels in GCF were significantly higher at sites positive for *A. actinomycetemcomitans* than at sites negative for this microorganism ($p<0.001$). GCF antibody titres tended to be higher at sites that harboured *P. gingivalis*, *T. denticola* and *B. forsythus* compared to those that were free of these pathogens, but this observation failed to reach statistical significance.

In a similar manner, serum IgG titres (EU) and IgG avidity (M at ID_{50}) were compared between patients who were positive and negative for a pathogen at baseline (Table 41). It appeared that serum IgG titres to a pathogen were higher for patients who were positive rather than negative to this pathogen. This was statistically significant for *P.*

Table 38. Comparison of site-specific clinical indices between sites positive and negative for each pathogen at baseline.

N = 159	PD[§] (mm)	p-value	RAL[§] (mm)	p-value	BOP*	p-value	Sup*	p-value
P.g. absent (107)	6.1 ± 1.2	0.61	14.0 ± 2.1	0.95	80.0	0.82	64.5	0.41
present (52)	6.0 ± 1.0		14.0 ± 1.9		79.0		57.6	
A.a. absent (123)	6.0 ± 1.1	0.15	14.0 ± 2.1	0.79	77.0	0.12	58.0	0.03
present (36)	6.3 ± 1.3		13.9 ± 1.9		89.0		78.0	
P.i. absent (98)	6.1 ± 1.2	0.33	14.1 ± 2.1	0.33	82.0	0.48	63.0	0.74
present (61)	6.0 ± 1.0		13.8 ± 1.9		77.0		60.7	
T.d. absent (88)	6.0 ± 1.1	0.75	13.8 ± 2.1	0.18	82.0	0.49	62.5	0.95
present (71)	6.1 ± 1.1		14.2 ± 1.9		77.0		62.0	
B.f. absent (31)	6.1 ± 1.2	0.81	13.9 ± 2.5	0.73	84.0	0.54	42.0	<0.01
present (128)	6.0 ± 1.1		14.0 ± 1.9		79.0		67.0	

Table 39. Comparison of full-mouth clinical indices between patients positive and negative for each pathogen at baseline.

N = 42	PD[§] (mm)	p-value	AL[§] (mm)	p-value	BOP[§] (%)	p-value	Sites≥5mm[§]	p-value
P.g. absent (26)	4.4 ± 0.7	0.450	5.1 ± 1.0	0.350	72.0 ± 16.0	0.300	69.0 ± 27.0	0.580
present (16)	4.3 ± 0.5		4.9 ± 0.9		66.0 ± 20.0		65.0 ± 18.0	
A.a. absent (28)	4.5 ± 0.6	0.020	5.3 ± 0.9	0.003	74.0 ± 14.0	0.055	74.0 ± 24.0	0.005
present (14)	4.1 ± 0.5		4.5 ± 0.7		62.0 ± 21.0		55.0 ± 16.0	
P.i. absent (17)	4.3 ± 0.7	0.760	5.1 ± 1.0	0.630	69.0 ± 18.0	0.720	63.0 ± 24.0	0.240
present (25)	4.4 ± 0.6		5.0 ± 0.9		71.0 ± 18.0		71.0 ± 23.0	
T.d. absent (10)	4.2 ± 0.7	0.300	4.5 ± 0.7	0.020	74.0 ± 23.0	0.560	61.0 ± 19.0	0.220
present (32)	4.4 ± 0.6		5.2 ± 0.9		69.0 ± 16.0		70.0 ± 25.0	
B.f. absent (3)	3.6 ± 0.2	0.010	4.8 ± 1.2	0.740	67.0 ± 15.0	0.760	44.0 ± 6.0	0.004
present (39)	4.4 ± 0.6		5.1 ± 0.9		70.0 ± 18.0		70.0 ± 23.0	

Footnote for Tables 38 & 39

[§]Mean ± sd; *% of sites; BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

P-values compare (i) sites and (ii) patients being either negative or positive for a pathogen

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*.

Table 40. GCF IgG antibody titres in patients positive and negative for each pathogen at baseline.

N = 159	GCF IgG titres[§]	p-value
P.g. absent (107)	14.2 ± 9.0	0.670
present (52)	28.0 ± 22	
A.a. absent (123)	876.0 ± 873.0	<0.001
present (36)	9705.0 ± 7992.0	
P.i. absent (98)	4.4 ± 2.0	0.800
present (61)	2.4 ± 0.5	
T.d. absent (88)	2.6 ± 0.4	0.290
present (71)	10.6 ± 4.0	
B.f. absent (31)	1.2 ± 0.4	0.770
present (128)	2.1 ± 0.7	

GCF IgG titres are expressed as EU/30sec

[§]Mean ± SEM;

P-values compare sites being either negative or positive for a pathogen

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*.

Table 41. Serum IgG titres and IgG avidity in patients positive and negative for each pathogen at baseline.

N = 42	Serum IgG titres[§]	p-value	IgG avidity[§]	p-value
P.g. absent (26)	70 (20, 321)	0.048	0.40 (0.30, 0.56)	0.054
present (16)	163 (71, 4990)		0.56 (0.50, 0.65)	
A.a. absent (28)	34 (12, 89)	<0.005	0.39 (0.30, 0.55)	0.090
present (14)	582 (66, 9378)		0.46 (0.40, 0.78)	
P.i. absent (17)	138 (29, 459)	0.180	0.69 (0.60, 1.00)	0.250
present (25)	232 (106, 821)		0.92 (0.70, 1.00)	
T.d. absent (10)	10 (6, 74)	0.337	0.29 (0.20, 0.36)	0.275
present (32)	18 (7, 64)		0.32 (0.20, 0.51)	
B.f. absent (3)	14 (12, 23)	0.143	0.33 (0.30, 0.80)	1.000
present (39)	38 (15, 1067)		0.42 (0.30, 0.60)	

Serum IgG titres are expressed as ELISA units (EU)

IgG avidity is expressed as molarity (M) at ID_{50}

[§]Median (Q1, Q3);

P-values compare subjects being either negative or positive for a pathogen

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*.

gingivalis ($p<0.05$) and for *A. actinomycetemcomitans* ($p<0.005$). In addition, IgG avidity seemed to be higher for the individuals who harboured the microorganisms than for those who had no detectable microorganisms. Nevertheless, this observation failed to reach statistical significance for any of the bacteria, ($p=0.054$ for *P. gingivalis* and $p=0.09$ for *A. actinomycetemcomitans*).

Table 42 compares the changes in site-specific clinical indices and changes in site microflora irrespective of the two treatment strategies (Q-SRP and FM-SRP) between BAS and R2. At R2, sites where *A. actinomycetemcomitans* was not detected (coded as 1) showed significantly greater reduction in all clinical indices than sites that had an increase or no change in the prevalence of this pathogen (coded as 0) ($p<0.005$ for ΔPD, $p<0.001$ for ΔRAL, $p<0.05$ for ΔBOP and $p<0.01$ for ΔSup). A greater reduction in suppuration was also seen for sites that became negative for *B. forsythus* at R2 than those that did not change or became positive for this species ($p<0.05$).

There were no significant differences in the changes of GCF IgG titres between sites that turned negative to an organism post-treatment and those that had no change or increase in the prevalence of that pathogen after therapy (data not shown). Despite the trend for a greater reduction in GCF antibody titres to *A. actinomycetemcomitans* at sites where this organism was no longer detected at R2, this was not statistically significant ($p>0.05$).

The changes in clinical, immunological and microbiological parameters of all subjects irrespective of the treatment groups were compared between BAS and R2. The comparison of the changes in full-mouth clinical indices and the changes in patient prevalence of the pathogens demonstrated that there was a significantly greater reduction in the No. of sites $\geq$ 5mm for the subjects who became negative for *T. denticola* ($p<0.05$) (Table 43). Interestingly, patients who were no longer positive to a pathogen at R2 tended to have a greater reduction in serum IgG antibody titres to that pathogen than patients who had no change or increase in the prevalence of this pathogen (Table 44). This was statistically significant for *A. actinomycetemcomitans* ($p<0.05$) but did not reach significance for the other pathogens. Also, IgG avidity seemed to be higher for the patients that turned negative to *A. actinomycetemcomitans* at R2, but this was not statistically significant.

Table 42. Comparison between changes in site-specific clinical indices and changes in site microflora.

N=147		ΔPD§ (mm) (BAS-R2)	p-value	ΔRAL§ (mm) (BAS-R2)	p-value	ΔBOP* (BAS-R2)	p-value	ΔSup* (BAS-R2)	p-value
P.g.	0 (99)	2.8 ± 1.4	0.720	1.0 ± 1.0	0.055	73.0	0.980	62.0	0.700
	1 (48)	2.9 ± 1.1		1.3 ± 0.8		73.0		58.0	
A.a.	0 (115)	2.7 ± 1.2	<0.005	1.0 ± 0.9	<0.001	69.0	0.035	55.0	0.007
	1 (32)	3.4 ± 1.3		1.6 ± 0.7		88.0		81.0	
P.i.	0 (101)	2.9 ± 1.3	0.210	1.1 ± 1.0	0.480	71.0	0.540	61.0	0.760
	1 (46)	2.6 ± 1.1		1.0 ± 0.8		76.0		59.0	
T.d.	0 (85)	2.8 ± 1.3	0.480	1.0 ± 0.9	0.070	78.0	0.121	59.0	0.620
	1 (62)	2.9 ± 1.2		1.3 ± 0.9		66.0		63.0	
B.f.	0 (43)	2.7 ± 1.5	0.560	0.9 ± 1.1	0.220	72.0	0.903	46.5	0.025
	1 (104)	2.9 ± 1.2		1.2 ± 0.8		73.0		66.0	

§Mean ± sd; *No. of sites; BAS = baseline; R2 = reassessment 2

1 = reduction and 0 = no change or increase in the detection frequency of a pathogen at BAS-R2

P-values compare sites showing reduction (1) with sites showing no change or increase (0) in the detection frequency of a pathogen

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus.*

Table 43. Comparison between changes in full-mouth clinical indices and changes in the microflora of patients.

N=39		ΔPD§ (mm) (BAS-R2)	p-value	ΔAL§ (mm) (BAS-R2)	p-value	ΔBOP§ (%) (BAS-R2)	p-value	ΔSites≥5mm§ (BAS-R2)	p-value
P.g.	0 (25)	1.7 ± 0.7	0.850	1.1 ± 0.6	0.490	60.0 ± 17.0	0.360	56.0 ± 24.0	0.730
	1 (14)	1.7 ± 0.5		1.2 ± 0.4		54.0 ± 21.0		59.0 ± 18.0	
A.a.	0 (28)	1.8 ± 0.7	0.390	1.1 ± 0.5	0.940	61.0 ± 18.0	0.150	61.0 ± 23.0	0.056
	1 (11)	1.6 ± 0.5		1.1 ± 0.4		51.0 ± 19.0		48.0 ± 17.0	
P.i.	0 (25)	1.7 ± 0.5	0.970	1.2 ± 0.5	0.230	56.0 ± 18.0	0.370	55.0 ± 18.0	0.500
	1 (14)	1.7 ± 0.8		1.0 ± 0.5		61.0 ± 20.0		61.0 ± 28.0	
T.d.	0 (14)	1.5 ± 0.6	0.065	1.1 ± 0.7	0.610	55.0 ± 22.0	0.540	48.0 ±15.0	0.037
	1 (25)	1.9 ± 0.6		1.2 ± 0.4		59.0 ± 16.0		62.0 ± 24.0	
B.f.	0 (13)	1.5 ± 0.8	0.230	1.0 ± 0.7	0.550	55.0 ± 17.0	0.440	52.0 ±24.0	0.300
	1 (26)	1.8 ± 0.5		1.2 ± 0.4		59.0 ± 19.0		60.0 ± 21.0	

Table 44. Comparison between changes in serum IgG titres and avidity and changes in the microflora of patients.

N=39		IgG titres* Change (BAS-R2)	p-value	IgG avidity* Change (BAS-R2)	p-value
P.g.	0 (25)	14 (-21, 237)	0.07	-0.030 (-0.250, 0.050)	0.36
	1 (14)	96 (25, 3318)		-0.025 (-0.150, 0.180)	
A.a.	0 (28)	5 (-9, 36)	0.03	-0.040 (-0.160, 0.040)	0.08
	1 (11)	34 (10, 2650)		-0.290 (-0.450, 0.030)	
P.i.	0 (25)	19 (-42, 93)	0.21	0.030 (-0.220, 0.120)	0.84
	1 (14)	35 (-2, 593)		0.035 (-0.030, 0.120)	
T.d.	0 (14)	3 (0.0, 35)	0.63	0.000 (-0.080, 0.070)	0.36
	1 (25)	5 (0.5, 31)		-0.030 (-0.100, 0.040)	
B.f.	0 (13)	2 (-4, 898)	0.75	-0.070 (-0.200, 0.090)	0.93
	1 (26)	6 (-2, 70)		0.000 (-0.150, 0.040)	

Footnote for Tables 43 & 44

§Mean ± sd; *median (Q1, Q3); BAS = baseline; R2 = reassessment 2

1 = reduction and 0 = no change or increase in the detection frequency of a pathogen at BAS-R2

P-values compare patients showing reduction (1) with patients showing no change or increase (0) in the detection frequency of a species.

5.2 Discussion

5.2.1 PCR prevalence of putative periodontal pathogens

The prevalence of the five putative periodontal pathogens presented here is relatively consistent with the range of values reported by other studies (Ashimoto et al. 1996; Riggio et al. 1996; Meurman et al. 1997; Riggio et al. 1998; Umeda et al. 1998). However, the present findings show a lower detection frequency for *P. gingivalis* compared to other studies (Wahlfors et al. 1995; Ashimoto et al. 1996; Griffen et al. 1998; Umeda et al. 1998) and disagree with results of other reports (Darby et al. 2001).

Despite the fact that the above quoted studies utilised PCR as a method for detecting the organisms, several reasons could account for the differences in the detection frequencies of the bacteria. The reasons for these variations are not clear. Variations in the clinical and laboratory procedures with respect to sampling techniques, primers used, primer annealing temperatures and processing of the samples prior to PCR analysis may contribute to conflicting results. Differences due to patients' racial or geographical characteristics may exist (Umeda et al. 1998), and it is likely that the subgingival microbial flora is in a state of dynamic flux and therefore, a single sampling may not accurately reflect the flora.

Darby et al. (2001) examined a group of patients from the same population as in the present study, who had moderate to advanced chronic periodontitis. In the current study and in those of Darby et al. (2001) and Riggio et al. (1996, 1998) the PCR analysis of the plaque samples was carried out in the same laboratory, thus reducing laboratory variations among studies. In the present investigation the primers targeted the 16S rRNA nucleotide region of all bacteria tested, while in the studies of Darby et al. (2001) and Riggio et al. (1996, 1998) the primers targeted the leukotoxin gene of *A. actinomycetemcomitans*, the fimbrillin gene of *P. gingivalis*, and the 16S rRNA nucleotide region of the other bacteria. It must be emphasised however, that since the present data agree generally with the results reported by Riggio et al. (1996, 1998), operator variability may be an important source of variation in the collection and processing the plaque samples for PCR analysis.

5.2.2 Bacterial combinations

The site-based analysis gave a detection frequency of 30.2% for the combination of *P.gingivalis* and *B. forsythus* and 42.0% for the combination of *T. denticola* and *B. forsythus*. The three bacteria together, *P. gingivalis*, *T. denticola* and *B. forsythus*, were present in 13.8% of the sites. The prevalence of each organism on its own at a site-specific level was 32.7% for *P. gingivalis*, 44.7% for *T. denticola* and 80.5% for *B. forsythus*. Socransky et al. (1998) found that 10% of sites were positive for the coexistence of *P. gingivalis*, *T. denticola* and *B. forsythus*, which is similar to the present finding (13.8%). In addition, present data agree with findings by Gmür et al. (1989) who showed that *P.gingivalis* was almost always detected in samples which harboured *B. forsythus*, although the latter organism was detected more frequently on its own. It is obvious, however, that since *B. forsythus* has been found to be one of the most prevalent organisms in subgingival plaque of periodontitis patients (Ashimoto et al. 1996; Meurman et al. 1997; Umeda et al. 1998; Darby et al. 2000), this species will be statistically more likely to cluster with other bacteria that have a lower detection frequency.

5.2.3 Subgingival microflora before and after scaling and root planing

The present study detected all five putative periodontal pathogens at a relatively high frequency pre-treatment, confirming previous reports that these organisms are related to periodontal disease (Slots et al. 1980; Dzink et al. 1983; Loesche et al. 1985; Lai et al. 1987; Simonson et al. 1992).

Both treatment modalities resulted in marked reductions in the percentage of sites and the subjects positive for the five putative periodontal pathogens and this paralleled significant improvements in all clinical indices, confirming the findings of previous reports (Simonson et al. 1992; Hafström et al. 1994; Shiloah and Patters 1994; Haffajee et al. 1997b). The site-based analysis revealed that all tested microorganisms were significantly reduced at R1 and R2. In addition, significant reductions in the percentage of patients positive for most of the bacteria tested were seen after therapy. However, the majority of the organisms were still detected post-scaling but in significantly lower frequencies than baseline. This is in agreement with other reports which showed that scaling and root planing lowers the numbers of selected periodontal

pathogens, but is unlikely to eliminate these species from any subject (Cugini et al. 2000; Haffajee et al. 1997a).

Data presented here showed that the microbial benefits of both treatment strategies were maintained over a six-month period in a group of highly motivated patients with optimal plaque control. The percentage of sites that were initially free of a pathogen but which subsequently became positive for that organism after treatment was negligible for both treatment groups, and although this percentage was higher on a patient-based analysis, it was still considered to be low. Whether this finding reflects deterioration and infection of sites or patients with a pathogen at a later stage is debatable since, the selected organisms may have been present at levels below the detection threshold pre-treatment.

It has been shown that the improvement of the subgingival microflora after mechanical periodontal treatment is not dramatic and requires supportive periodontal therapy for its maintenance (Haffajee et al. 1997a). This finding disagrees with the present study, which shows that conservative periodontal therapy results in marked microbiological and clinical improvements. Reductions in the detection of the tested organisms presented here exceed those reported by Darby et al. (2001) who found significant reductions in *P. gingivalis*, *P. intermedia* and *B. forsythus* on a site-basis and little change in the microflora on a subject-basis. However, differences in the clinical and immunological procedures and parameters between the two studies also need to be considered in order to explain the inconsistency in the microbiological findings.

The results of the current study do not agree with the models of microbial re-population, proposed by Slots et al. (1979). The current study revealed that scaling and root planing resulted in a rapid reduction of the gram-negative anaerobic microorganisms, including *Bacteroides* species but in contrast to the findings of Slots et al. (1979), this improvement was not followed by a return that exceeded pre-treatment levels. However, it must be emphasised that the present study compared the detection frequencies of the tested organisms before and after treatment, while the study of Slots et al. (1979) compared the bacterial proportions and counts. On the other hand, the current data support the findings of another study which demonstrated a decrease in the prevalence of *P. gingivalis*, *A. actinomycetemcomitans* and *P. intermedia* 12 months after scaling and root planing in the absence of supportive periodontal therapy (Shiloah

and Patters, 1996). It has been shown in another report, that the greatest clinical improvement and greatest reductions in selected subgingival organisms occurred within the first six months post-scaling which agrees with the findings of the current study, and that both clinical and microbiological parameters remained stable or improved moderately afterwards (Cugini et al. 2000).

Several studies have shown that conventional periodontal therapy is ineffective at reducing the levels of *A. actinomycetemcomitans* (Kornman and Robertson, 1985; Mombelli et al. 1994b), and there is evidence that its presence post-treatment is related to a compromised clinical outcome (Slots et al. 1986; Renvert et al. 1990a). In addition, there are data demonstrating that the proportions of this organism increase after treatment (Renvert et al. 1990a; Pedrazzoli et al. 1992), possibly as a result of higher sensitivity of the other subgingival species to debridement. The present study showed a marked decrease in the prevalence of this organism at a site-specific level, but despite the reduction in the percentage of patients that possessed this pathogen after treatment, this was not found to be statistically significant.

It was interesting to note that patients who were still positive for any of the tested organisms at six months did not appear to have higher indices for pocket depth, attachment level or bleeding score than those who did not. This finding is not consistent with findings of Renvert et al. (1996), who showed that patients who lost attachment or returned to pre-treatment levels after an initial gain harboured *P. gingivalis*, *A. actinomycetemcomitans* and *P. intermedia* more frequently than those who maintained their initial attachment gain. It should be noted, that the current study followed the patients over a six-month period of time, whereas the previous investigation was a longer-term study that lasted for five years. Present results agree with those by Doungudomdacha et al. (2001), who showed a significant decrease but not eradication of the tested organisms post-treatment. The presence of organisms at sites that were considered clinically healthy implied that the mere presence of a putative pathogen is not indicative of presence or recurrence of disease.

5.2.4 Comparison of microbiological responses between Q-SRP and FM-SRP groups

T. denticola was more prevalent in the FM-SRP patients at baseline. The patient-based analysis showed that although there were no significant differences in the detection of *T. denticola* between the two treatment groups at R1 and R2, a greater reduction for this organism was seen in the FM-SRP group than in the Q-SRP group between BAS and R1 and BAS and R2. However, it is difficult to interpret this finding, given the unbalanced distribution of *T. denticola* between the two treatment groups at baseline.

FM-SRP resulted in significantly lower percentages of sites positive for *P. intermedia* at R1 than did Q-SRP. This was also the case on a subject-based analysis. The lower prevalence of FM-SRP patients positive for *P. intermedia* and *T. denticola* at R1, albeit statistically significant only for *P. intermedia*, paralleled the significantly greater reduction in serum antibody levels to these pathogens between BAS and R1 for the FM-SRP group compared to the Q-SRP group. This is in agreement with other studies showing that reductions in the serum antibody levels post-treatment reflect reductions in the antigenic load (Mouton et al. 1987; Aukhil et al. 1988; Murray et al. 1989).

In addition, selected sites of FM-SRP patients showed less PD reduction between BAS and R1 than those of Q-SRP patients, indicating that at six weeks, post-scaling healing is still occurring and that the immunological and microbiological improvements may precede clinical changes. These results show that in the short-term (BAS-R1), significant differences in clinical, immunological and microbiological parameters were found between the two treatment groups, but these differences seemed to disappear at six months.

In the present study, marked microbiological improvements for all tested organisms were seen after both treatments. Nevertheless, except for the short-term differences between the two treatment groups at R1, there were no significant differences in the detection of any organism at R2. Therefore, these data do not confirm the previous findings of Quirynen et al. (2000), which showed that over a period of eight months FM-SRP with and without the use of chlorhexidine resulted in a less pathogenic microflora compared to the common therapy of consecutive sessions of Q-SRP. That study also showed that at eight months the number of CFU/ml of specific periodontal

pathogens returned to pre-treatment values only for the Q-SRP group. Although the present results are based on detection frequencies of selected pathogens, no such deterioration was noted for the Q-SRP group at six months. A later study by the same investigators showed a greater reduction in the prevalence and levels of the "red" and "orange" complex species (Table 3, section 1.5.6), but only for the chronic periodontitis patients who received the one-stage full-mouth disinfection treatment (De Soete et al. 2001). The present microbiological data were analysed differently and therefore no comparisons can be made between the two studies in this respect.

5.2.5 Re-colonisation of treated sites by pathogens from the remaining untreated periodontal pockets

The rationale behind the one-stage full-mouth disinfection was to prevent re-colonisation of the treated sites by periodontal pathogens from the remaining untreated pockets and other intra-oral niches and therefore prevent any recurrence of disease (Quirynen et al. 2001). The one-stage full-mouth scaling and root planing with no use of chlorhexidine was shown to be equally efficacious with the one-stage full-mouth disinfection therapy, and more beneficial than the classical treatment of consecutive sessions of scaling and root planing in terms of clinical and microbiological responses (Quirynen et al. 2000). These findings indicate that the primary source of bacterial translocation are the periodontal pockets. The authors suggest that these considerations should be taken into account when split-mouth clinical trials are designed. Another study from the same laboratory demonstrated that the composition of the microflora around teeth significantly influenced the formation of the subgingival flora around implants, and this was more pronounced when teeth and implants were in the same jaw (Quirynen et al. 1996). This finding highlights the role of the periodontal pockets as bacterial reservoirs.

The clinical design of the present study consisted of consecutive sessions of scaling and root planing at two-weekly intervals for the Q-SRP group, with no use of antiseptics during or after treatment. Q-SRP consistently started with quadrant one and continued clockwise with quadrant two, three and four every second week. The comparison of clinical and microbiological effects of Q-SRP on quadrants one and four showed that the clinical outcome and improvements in the microbial flora were similar for both quadrants post-treatment. These findings imply that there was no recurrence of disease

or re-colonisation of the already treated sites (1Q) by specific periodontal pathogens during a healing period of six months.

However, there is evidence for enhanced clinical outcome after full-mouth versus partial-mouth periodontal therapy (Mombelli et al. 1996; Nowzari et al. 1996), which supports the hypothesis posed by Quirynen et al. (1995). Mombelli et al. (1996) showed that patients who received local treatment at the test sites only, had less pocket depth reduction than those who received full-mouth treatment. However, the former group of patients received no oral hygiene instructions and had plaque indices higher than the latter group of patients. Therefore, the compromised clinical outcome seen in these patients could be a result of ineffective plaque control.

Nowzari et al. (1996) showed that patients who had no pockets $\geq$ 5 mm (other than the study sites), and low levels of periodontopathogens, had lower levels of pathogens in membranes used for guided tissue regeneration and higher clinical attachment gains compared to patients with multiple deep sites and high levels of pathogens. Nevertheless, it is unclear whether the recovered organisms from the membranes were true contaminants transmitted from other periodontal pockets. One supposition is that the microbial contamination of the membranes occurred during membrane manipulation prior to application, and originated from the skin or from other areas in the mouth. In addition, the recovered pathogens from the membranes may originate from the test sites themselves, a possibility which was not assessed by the investigators since, prior to regenerative therapy, microbial sampling was performed at sites other than the test pockets.

In contrast, in a cariology study, Axelsson et al. (1987) examined the full-mouth disinfection treatment compared to another two treatment approaches which consisted of less intensive prophylactic measures. This study showed that no long-term differences in the reduction of *S. mutans* and caries existed between the various treatments, implying that the additional full-mouth preventive measures used were no more successful in preventing caries progression than the less intensive prophylactic measures.

Translocation of microorganisms from one site to another may occur *via* saliva (van Winkelhoff et al. 1988), oral hygiene aids (Svanberg, 1978; Glass and Lare, 1986; Müller et al. 1989), and dental instruments (Loesche et al. 1973, 1979; Barnett et al. 1981; Preus et al. 1993; Papaioannou et al. 1996). Suspected periodontal pathogens were also found in supragingival plaque deposits (Riviere et al. 1992; Ximénez-Fyvie et al. 2000b; Haffajee et al. 2001b), indicating the possibility that they may be translocated to other intra-oral areas. Although, pathogens were recovered following the transmission processes mentioned above, it remains debatable whether inoculation of microorganisms takes place into sites currently free of these species. It is unlikely that inoculation of organisms occurs *via* saliva since, the constant outflow of GCF from the pockets prevents this from happening. Christersson et al. (1985) demonstrated that despite the fact that periodontal probes could transmit *A. actinomycetemcomitans* from infected to uninfected sites in localised aggressive periodontitis patients, this organism was eliminated from the recipient sites within three weeks. It has been shown that a combination of factors such as changes in the local environment, presence of virulent strains of periodontal pathogens in sufficient numbers, absence of beneficial species and host susceptibility contribute to disease initiation or recurrence (Socransky and Haffajee, 1992, 1993). In agreement with this are findings from other studies which detected suspected periodontal pathogens in sites that were considered clinically healthy, suggesting that mere colonisation does not necessarily mean induction of clinical signs of disease (Nieminen et al. 1995; Doungudomdacha et al. 2001).

In conclusion, the current study does not provide evidence of microbiological and clinical deterioration at sites that were root planed six weeks before the completion of the Q-SRP treatment or at sites that received Q-SRP treatment over several visits compared to those that were treated with one-day FM-SRP. In the present study, FM-SRP was completed in 12 hours instead of 24 hours as described by Quirynen et al. (1995). Although the time interval between initiation and completion of treatment was lessened, and therefore the chances for bacterial re-colonisation were also reduced, no microbiological or clinical differences were found between the two treatment groups six months post-scaling to support the hypothesis of Quirynen et al. (1995). It must be stressed, however, that the participants in this study were highly motivated patients and practised a high standard of plaque control measures. Several studies showed that meticulous plaque control can affect the clinical and microbiological parameters in

moderate (McNabb et al. 1992; Ximénez-Fyvie et al. 2000a), and deep pockets (Smulow et al. 1983; Dahlén et al. 1992; Hellström et al. 1996). These data emphasise the magnitude of oral hygiene measures on the subgingival environment. Haffajee et al. (2001b) suggested that the beneficial effects of supragingival plaque control on the composition of the supra- and subgingival microflora decreased the risk of disease initiation or recurrence in maintenance periodontitis patients.

Taking these factors into consideration, although there is a potential for translocation of periodontal pathogens from one pocket to another, carefully performed plaque control measures and changes in the subgingival environment and the host response induced by treatment, may prevent re-infection of sites, and thus induction or relapse of periodontal disease.

5.2.6 Effect of the presence and absence of putative periodontal pathogens on clinical and immunological parameters

The data reported in the current study showed that suppuration was significantly higher at sites positive for *A. actinomycetemcomitans* and *B. forsythus* than sites negative for these pathogens. Sites positive or negative for a pathogen had similar pocket depths, relative attachment levels and bleeding scores. This could be due to the fact that the selected sites were all relatively deep (mean PD >6.0 mm) and were unable to show any differences in clinical and immunological parameters based on the presence or absence of an organism. However to confirm this, the microbiological and immunological parameters of selected sites were analysed based on pocket depth. The results of this analysis will be discussed in Chapter seven.

The subject-based analysis showed lower pocket depths and attachment levels and fewer pockets with PD $\geq$ 5 mm in subjects positive for *A. actinomycetemcomitans*, while subjects positive for *T. denticola* had higher attachment levels and those positive for *B. forsythus* had deeper pockets and more pockets with PD $\geq$ 5mm. These findings may reflect the fact that *A. actinomycetemcomitans* is a facultative anaerobe, whereas *T. denticola* and *B. forsythus* are fastidious anaerobes, thriving in more advanced periodontal lesions (Simonson et al. 1988; Gmür et al. 1989; Kigure et al. 1995; Socransky et al. 1998; Ximénez-Fyvie et al. 2000b). The data presented in the current study confirm the findings of Wolff et al. (1985), who showed that the proportions of *A.*

actinomycetemcomitans were higher in pockets of moderate depth and disagreed with the findings of Slots et al. (1980), who demonstrated that this pathogen was positively related to pocket depth. One possible explanation for this disagreement could be that the present study and the study by Wolff et al. (1985) examined patients with moderate to advanced periodontitis, in contrast to the study of Slots et al. (1980). The present study does not confirm previous findings that *P. gingivalis* is elevated in deep pockets (Gmür et al. 1989; Socransky et al. 1991b; Kigure et al. 1995; Ximénez-Fyvie et al. 2000b), and that this organism is more prevalent at suppurating sites (Tanner et al. 1979; Socransky et al. 1991b).

GCF antibody titres were significantly higher at sites positive for *A. actinomycetemcomitans* and tended to be elevated at sites that harboured *P. gingivalis*. Serum IgG titres and avidities followed the same pattern of elevated levels in the subjects that harboured the tested organisms. However, this was statistically significant for serum IgG titres in patients positive for *P. gingivalis* and *A. actinomycetemcomitans*. These findings support earlier reports that the presence of elevated antibody titres in periodontitis patients is related to bacterial infection (Ebersole et al. 1982a; Choi et al. 1990; Nakagawa et al. 1990; Kojima et al. 1997), and imply that the production of antibodies of elevated avidity is a result of repeated antigenic exposure (Lopatin et al. 1991), as has been discussed in Chapter four.

Patients were subdivided into two groups, those who became negative for a bacterial species at R2 and those who remained the same or became positive for this species. Then the changes in clinical and immunological parameters between BAS and R2 were compared between these two groups of patients. A greater decrease in the number of sites $\geq$ 5 mm was found in subjects who became negative for *T. denticola* than those who showed no change or became colonised by this organism. These results highlight that a reduction in prevalence of specific periodontal pathogens with treatment parallels an improved clinical outcome (Slots et al. 1979; Simonson et al. 1992; Mombelli et al. 1994b). On a site-based analysis, sites that became negative for *A. actinomycetemcomitans* after treatment were found to have a greater reduction in all clinical indices than sites that demonstrated no change or an increase in the prevalence of this pathogen. A trend for greater gain in RAL which was close to statistical

significance was seen at sites that were no longer positive for *P. gingivalis* and *T. denticola* following treatment.

A greater reduction in serum IgG antibody titres to *A. actinomycetemcomitans* was found in subjects who became negative for *A. actinomycetemcomitans* at R2 than those who showed no change or became colonised by this pathogen. Although, the other organisms followed the same trend, this observation failed to show statistical significance. This finding is consistent with other reports that associated post-scaling reductions in antibody titres with reductions in the microbial burden (Mouton et al. 1987; Aukhil et al. 1988; Murray et al. 1989; Choi et al. 1990). The local antibody responses failed to show significant differences in GCF antibody titres between sites that became negative for an organism and those that showed no change or became positive for that species.

CHAPTER 6

IMPACT OF SMOKING ON PERIODONTAL PARAMETERS

6. Impact of smoking on periodontal parameters

6.1 Results

6.1.1 Effect of smoking on all subjects (n=42). Comparison of baseline clinical, immunological and microbiological parameters between non-smokers and smokers

Table 45, compares the clinical indices in 17 smokers and 25 non-smokers at baseline. Clinical indices collected from the selected sites of smokers showed significantly lower PD ($p<0.05$) and MGI ($p=0.005$) at baseline compared to sites of non-smokers. Smokers tended to have greater RAL and lower BOP, but this failed to reach statistical significance. In addition, no significant differences were noted in PI and in the whole-mouth clinical indices between the two subgroups at baseline.

Although serum IgG titres (EU) to all putative pathogens were lower for smokers at baseline, this observation failed to reach statistical significance (Table 46). No significant differences in IgG avidity (M at ID_{50}) were seen between non-smokers and smokers (Table 47). Table 48 compares the GCF IgG titres (EU / 30 sec) and GCF volume (µl / 30 sec) between smokers and non-smokers at baseline and shows significantly lower GCF volume for smokers ($p<0.01$). In general, GCF IgG titres were lower for smokers than non-smokers at baseline and this was significant for *A. actinomycetemcomitans* ($p<0.005$) and *P. intermedia* ($p<0.01$) and just failed to reach statistical significant for *P. gingivalis* ($p=0.057$).

Despite the differences between smokers and non-smokers in the proportions of patients and sites positive for the five putative periodontal pathogens, none of these differences reached statistical significance (Table 49).

Cigarette smoking status was self-reported by the patients. A retrospective serum cotinine enzyme inhibition assay confirmed this self-reporting to be reliable (Figure 15). Subjects were considered to be smokers if they smoked five or more cigarettes *per* day. Since, no participants smoked less than five cigarettes *per* day, less than five cigarettes *per* day seemed to be a useful gap in the diagnostic differentiation of smokers versus non-smokers. Three patients (one in Q-SRP group and two in FM-SRP group) reported that they had given up smoking after the initiation of periodontal therapy, but these subjects were included in the smoking subgroup.

Table 45. Comparison of baseline whole mouth and site-specific clinical indices between smokers and non-smokers (all subjects).

whole mouth	PD (mm)	AL (mm)	BOP (%)	No. sites≥5 mm	Site-specific	PD (mm)	RAL (mm)	BOP*	Sup*	MGI	PI
Non-smokers	4.4 ± 0.6	4.9 ± 0.8	71.0 ± 19.0	68.0 ± 21.0	Non-smokers	6.2 ± 1.3	13.8 ± 2.2	85.0	62.0	2.6 ± 0.8	2.0 ± 0.7
Smokers	4.4 ± 0.8	5.2 ± 1.1	69.0 ± 16.0	67.0 ± 27.0	Smokers	5.8 ± 0.8	14.2 ± 1.7	73.5	65.0	2.3 ± 0.5	1.9 ± 0.7
p-value	0.900	0.300	0.800	0.900	p-value	0.016	0.200	0.066	0.700	0.005	0.650

Non-smokers = 25; smokers = 17; sites of non-smokers = 100; sites of smokers = 68

Mean ± sd; *% of positive sites

PD = pocket depth; AL = attachment level, RAL = relative attachment level; BOP = bleeding on probing; Sup = suppuration; MGI = modified gingival index; PI = plaque index.

Table 46. Comparison of baseline serum IgG titres between smokers and non-smokers (all subjects).

	P.g.	*A.a*	*P.i.*	*T.d*	*B.f.*
Non-smokers (25)	109 (54, 716)	73 (20, 1536)	277 (128, 663)	15 (8, 77)	34 (15, 975)
Smokers (17)	59 (17, 469)	49 (10, 95)	94 (23, 640)	12 (3, 47)	28 (13, 876)
p-value	0.15	0.08	0.10	0.30	0.80

Serum IgG titres are expressed as ELISA units (EU)

Median (Q1, Q3)

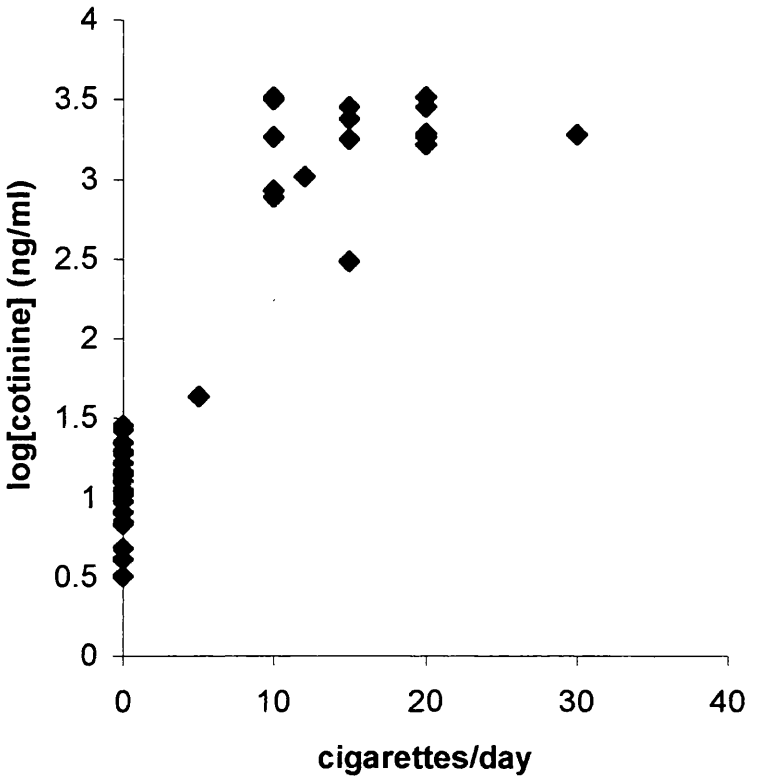
P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*.

Table 49. Percentage of patients and sites positive for the five putative periodontal pathogens in smokers and non-smokers in all subjects at baseline.

Positive patients	*P.g.*	*A.a*	*P.i.*	*T.d*	*B.f.*	Positive sites	*P.g.*	*A.a*	*P.i.*	*T.d*	*B.f.*
Non-smokers (25)	36.0	40.0	68.0	76.0	96.0	Non-smokers (97)	33.0	27.0	42.3	44.3	82.4
Smokers (17)	41.0	23.5	47.0	76.5	88.0	Smokers (62)	32.3	16.0	32.3	45.2	77.5
p-value	0.7	0.3	0.2	1.0	0.6	p-value	0.9	0.1	0.2	0.9	0.4

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*.

Figure 15. Cotinine verification of smoking status.



The assay was performed using the COZART serum cotinine assay kit (Abingdon, U.K.), following the manufacturer's instructions. In brief, all reagents were warmed to room temperature. 10 µl dilutions of cotinine standards and serum (neat and 1/5 dilution) were added to micro-plate wells in duplicate. Then 100 µl of cotinine enzyme was added to each well and the reaction was incubated for 30 min at room temperature. The plate was washed four times with wash buffer and 100 µl of substrate reagent was added to each well and the plate was incubated for 30 min at room temperature. The reaction was terminated with 100 µl of stop solution and optical densities were read using a Dynex Technologies MRX II plate reader at 450 nm with a 630 nm reference wavelength. Sample readings were compared with a reference line derived from serial dilutions of the cotinine standards. The median concentration of cotinine was significantly lower in non-smokers (11.2 ng/ml; range: 3.2-28.6) than smokers (1848.0 ng/ml; range: 43.0-3267.0); Mann Whitney test $p<0.001$. Therefore, self-reported smoking status was confirmed.

In the following sections the clinical, immunological and microbiological parameters and their change with treatment will be examined for smokers and non-smokers in each treatment group.

6.1.2 Effect of smoking on clinical, immunological and microbiological parameters in the Q-SRP group

Seven smokers and 13 non-smokers were included in the Q-SRP group. Table 50, summarises the analysis of the whole-mouth clinical indices and it shows significantly greater BOP reduction for non-smokers at R1 and R2 from BAS compared to smokers ($p<0.05$).

The site-specific clinical indices between non-smokers and smokers before and after Q-SRP were also determined (Table 51). Selected sites of smokers showed significantly lower gingival inflammation (BOP and MGI) and a trend for lower PD and greater RAL at baseline. BOP was present in 64% of sites in smokers versus 92% in non-smokers ($p<0.005$) and mean MGI was 2.2 in smokers versus 2.7 in non-smokers ($p=0.005$). No significant differences in PI and suppuration were found between the two subgroups. The analysis of the changes in site-specific clinical indices with treatment showed significantly less clinical improvement for smokers (ΔPD, ΔRAL, ΔBOP). There was a greater PD reduction of approximately 1.2 mm for non-smokers at R1 and R2 from BAS ($p<0.001$). A greater gain in RAL of 0.6 mm was found for non-smokers at R1 and R2 from BAS compared to smokers ($p<0.01$). Similarly, there was a greater reduction in BOP by approximately 30% and 51% for non-smokers than smokers at R1 and R2 from BAS, respectively ($p<0.01$). 14% of sites in smokers that were negative for BOP at baseline turned positive at R2, but no sites in non-smokers did so. Despite the fact that smokers had less clinical improvement in comparison with non-smokers, they still had a significant reduction in all clinical indices following treatment ($p<0.05$).

The serum IgG titres (EU) and IgG avidity (M at ID_{50}) were compared between smokers and non-smokers in the Q-SRP group and the results are shown in Tables 52 and 53. At baseline, serum IgG titres tended to be lower for smokers (Table 52). This was statistically significant for *P. intermedia* ($p<0.05$), and just failed to reach statistical significance for *A. actinomycetemcomitans* ($p=0.052$). After treatment, despite the lower levels of IgG antibody for smokers at R1 and R2 (significantly for *A.*

Table 50. Comparison of whole-mouth clinical indices between smokers and non-smokers before and after Q-SRP.

$N_{non\text{-}smokers}$=13 $N_{smokers}$=7	Baseline	p-value[2]	R1	R2	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
PD(mm) non-smokers	4.4 ± 0.6	0.70	2.7 ± 0.3	2.5 ± 0.3	1.8 ± 0.7	<0.001	0.330	1.9 ± 0.7	<0.001	0.23
smokers	4.3 ±0.8		2.8 ± 0.4	2.8 ± 0.3	1.5 ± 0.5	<0.001		1.6 ± 0.5	<0.001	
AL(mm) non-smokers	5.0 ± 0.8	0.75	3.9 ± 0.9	3.9 ± 0.9	1.1± 0.5	<0.001	0.860	1.1 ± 0.6	<0.001	0.86
smokers	4.9 ± 1.0		3.8 ± 1.0	3.8 ± 1.0	1.1 ± 0.5	0.002		1.1 ± 0.5	0.001	
BOP(%) non-smokers	75.0 ± 19.0	0.17	16.0 ± 8.0	11.6 ± 5.4	59.0 ± 19.0	<0.001	0.047	64.0 ± 19.0	<0.001	0.04
smokers	63.0 ± 17.0		19.0 ± 10.0	16.7 ± 8.4	44.0 ± 12.0	<0.001		47.0 ± 14.0	<0.001	
No. sites≥5 mm										
non-smokers	72.0 ± 18.0	0.49	12.0 ± 9.0	8.5 ± 8.1	60.0 ± 20.0	<0.001	0.320	63.5 ± 20.0	<0.001	0.35
smokers	64.0 ± 25.0		14.0 ± 17.0	10.0 ± 10.0	50.0 ± 20.0	0.001		54.5 ± 20.0	<0.001	

Mean ± sd

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

PD = pocket depth; AL = attachment level; BOP = bleeding on probing

[1]P-value is given for changes in non-smokers and smokers before/after Q-SRP

[2]P-value compares differences between non-smokers and smokers.

Table 51. Comparison of site-specific clinical indices between smokers and non-smokers before and after Q-SRP.

$N_{non\text{-}smokers}$=52 $N_{smokers}$=28		Baseline	p-value[2]	R1	R2	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
PD(mm)	non-smokers	6.4 ± 1.3	0.110	3.3 ± 1.0	3.1 ± 0.7	3.2 ± 1.3	<0.001	<0.001	3.3 ± 1.3	<0.001	<0.001
	smokers	5.9 ± 1.0		4.1 ± 1.2	3.7 ± 0.7	1.8 ± 1.1	<0.001		2.2 ± 0.9	<0.001	
RAL(mm)	non-smokers	13.8 ± 2.4	0.230	12.8 ± 2.3	12.6 ± 2.2	1.0 ± 0.9	<0.001	0.004	1.2 ± 0.8	<0.001	0.004
	smokers	14.4 ± 2.0		14.0 ± 2.1	13.8 ± 2.0	0.4 ± 0.9	0.040		0.6 ± 0.9	0.001	
BOP*	non-smokers	92.0	0.004	17.0	6.0	75.0	<0.001	0.006	86.5	<0.001	<0.001
	smokers	64.0		18.0	29.0	46.0	0.001		35.7	0.031	
Sup*	non-smokers	63.5	0.580	4.0	2.0	60.0	<0.001	0.490	61.5	<0.001	0.580
	smokers	57.0		3.6	0.0	53.6	<0.001		57.0	<0.001	
MGI	non-smokers	2.7 ± 0.9	0.005	0.5 ± 0.5	0.1 ± 0.3	2.2 ± 1.0	<0.001	0.300	2.6 ± 1.0	<0.001	0.260
	smokers	2.2 ± 0.5		0.4 ± 0.6	0.3 ± 0.4	1.8 ± 0.7	<0.001		1.9 ± 0.5	<0.001	
PI	non-smokers	1.9 ± 0.8	0.770	0.9 ± 0.8	0.8 ± 0.7	1.0 ± 0.8	<0.001	0.330	1.2 ± 0.9	<0.001	0.330
	smokers	2.0 ± 0.7		0.8 ± 0.8	0.6 ± 0.6	1.2 ± 0.7	<0.001		1.4 ± 0.7	<0.001	

Mean ± sd; *% of positive sites;

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

PD = pocket depth; RAL = relative attachment level; BOP = bleeding on probing; Sup = suppuration; MGI = modified gingival index; PI = plaque index

[1]P-value is given for changes before/after Q-SRP for non-smokers and smokers; [2]p-value is given for differences between non-smokers and smokers.

Table 52. Serum IgG titres in smokers and non-smokers before and after Q-SRP.

$N_{non\text{-}smokers}$ =13 $N_{smokers}$ =7	Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g. non-smokers	175 (55, 1566)	0.360	81 (41, 1917)	0.300	77 (37, 958)	0.240	-3 (-146, 87)	0.200	90 (-11, 610)	0.800
smokers	70 (9, 516)		76 (5, 255)		39 (8, 228)		42 (0, 261)		31 (1, 391)	
A.a. non-smokers	95 (29, 1490)	0.052	96 (34, 1041)	<0.030	58 (31, 555)	0.009	12 (-27, 387)	0.500	15 (-14, 14)	0.600
smokers	21 (5, 67)		12 (5, 58)		21 (4, 25)		2 (0, 11)		2 (-5, 34)	
P.i. non-smokers	223 (104, 792)	0.048	188 (102, 856)	0.070	136 (85, 511)	0.057	30 (-137, 121)	0.300	32 (-21, 475)	0.500
smokers	60 (20, 162)		43 (24, 173)		42 (24, 129)		2 (-39, 6)		6 (-16, 70)	
T.d. non-smokers	25 (8, 140)	0.088	27 (8, 133)	0.060	29 (8, 80)	0.052	2 (-37, 4)	0.500	3 (-5, 71)	0.700
smokers	4 (1, 14)		3 (2, 19)		1 (1, 14)		-1 (-5, 2)		2 (0, 3)	
B.f. non-smokers	38 (19, 1426)	0.600	0.4 (0.3, 0.6)	0.900	0.4 (0.3, 0.7)	0.900	-3 (-52, 303)	0.500	2 (-8, 310)	0.300
smokers	28 (12, 117)		0.4 (0.3, 0.5)		0.4 (0.2, 1.1)		1 (-6, 88)		19 (0, 63)	

Serum IgG titres are expressed as ELISA units (EU)

Median (Q1, Q3)

P-values are given for differences between non-smokers and smokers

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

actinomycetemcomitans, $p<0.05$ and close to statistical significance for *P. intermedia* $p=0.057$ and for *T. denticola* $p=0.052$), the comparison of the changes in antibody titres between BAS and R1 and BAS and R2 were not significantly different between smokers and non-smokers. IgG avidity was similar for smokers and non-smokers at baseline, and no significant differences in antibody avidity to any microorganism were noted at R1 and R2 (Table 53). When the changes in IgG avidity were examined, no significant differences in IgG avidity for any microorganism were noted between smokers and non-smokers at R1 and R2 from BAS.

The levels of statistical significance for the changes in the serum immunological parameters in non-smokers and smokers post Q-SRP are summarised in Table 54. The comparison of changes in serum IgG titres before and after Q-SRP for the two subgroups showed significant decrease in antibody titres to *P. gingivalis* for non-smokers ($p<0.05$) and smokers ($p<0.05$) after treatment. The decrease in serum antibody titres to *B. forsythus* found at R2 for smokers was close to statistical significance ($p=0.06$). No significant changes in IgG avidity were noted for each subgroup after treatment.

The analysis of GCF volume (µl / 30 sec) between non-smokers and smokers is shown in Table 55. It is evident that non-smokers had significantly higher GCF volume at baseline ($p<0.05$). In addition, a significantly greater reduction in GCF volume was seen for non-smokers between BAS and R2 ($p=0.01$). However, both non-smokers and smokers showed significant reductions in GCF volume after treatment ($p<0.05$). At baseline, GCF IgG titres (EU / 30 sec) were lower in the smokers compared with the non-smokers (Table 56). This finding was significant for *P. gingivalis* ($p=0.01$), *A. actinomycetemcomitans* ($p<0.005$), *P. intermedia* ($p<0.005$) and *T. denticola* ($p<0.05$). When the changes in GCF IgG titres with treatment were examined there was a trend for a greater reduction in non-smokers and this finding was statistically significant for *T. denticola* at R1 ($p<0.05$) and R2 ($p<0.005$) from baseline.

The prevalence of the five putative periodontopathogens between non-smokers and smokers before and after Q-SRP is depicted in Tables 57 and 58. Despite the differences in the prevalence of pathogens between the two subgroups before and after treatment, no statistically significant differences were found for any microorganism on a

Table 53. IgG avidity in smokers and non-smokers before and after Q-SRP.

$N_{non\text{-}smokers}$ =13 $N_{smokers}$ =7	Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g. non-smokers	0.47 (0.30, 0.70)	0.66	0.49 (0.30, 0.90)	0.12	0.55 (0.30, 0.90)	0.90	-0.05 (-0.30, 0.05)	0.14	-0.07 (-0.30, 0.06)	0.90
smokers	0.41 (0.30, 0.60)		0.40 (0.30, 0.50)		0.39 (0.30, 1.30)		0.04 (-0.03, 0.10)		-0.04 (-0.90, 0.17)	
A.a. non-smokers	0.42 (0.30, 0.70)	0.22	0.42 (0.30, 0.60)	0.55	0.43 (0.30, 0.80)	1.00	0.0 (-0.06, 0.09)	0.80	0.00 (-0.20, 0.04)	0.70
smokers	0.34 (0.30, 0.50)		0.33 (0.20, 0.50)		0.41 (0.30, 0.70)		0.03 (-0.08, 0.10)		-0.08 (-0.30, 0.07)	
P.i. non-smokers	0.80 (0.60, 1.00)	0.75	0.71 (0.70, 0.90)	0.72	0.78 (0.70, 1.00)	0.40	0.06 (-0.09, 0.30)	1.00	0.00 (-0.10, 0.08)	0.97
smokers	0.91 (0.50, 1.30)		0.84 (0.50, 1.20)		1.03 (0.70, 1.20)		0.09 (-0.14, 0.40)		0.03 (-0.20, 0.20)	
T.d. non-smokers	0.30 (0.20, 0.40)	0.78	0.33 (0.30, 0.50)	0.69	0.34 (0.30, 0.40)	0.30	-0.03 (-0.20, 0.02)	0.78	-0.03 (-0.10, 0.05)	0.72
smokers	0.30 (0.20, 0.40)		0.28 (0.20, 0.70)		0.25 (0.20, 0.40)		-0.07 (-0.40, 0.06)		-0.03 (-0.10, 0.00)	
B.f. non-smokers	0.40 (0.30, 0.70)	0.11	0.38 (0.30, 0.60)	0.90	0.37 (0.30, 0.70)	0.90	0.02 (-0.10, 0.16)	0.07	0.02 (-0.10, 0.10)	0.18
smokers	0.29 (0.20, 0.50)		0.43 (0.30, 0.50)		0.38 (0.20, 1.10)		-0.12 (-0.20, 0.00)		-0.07 (-0.80, 0.01)	

IgG avidity is expressed as molarity (M) at ID_{50}

Median (Q1, Q3)

P-values are given for differences between non-smokers and smokers

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 54. P-values for changes in serum IgG titres and IgG avidity in non-smokers and smokers after Q-SRP.

IgG titres	p-value (BAS-R1)	p-value (BAS-R2)	IgG avidity	p-value (BAS-R1)	p-value (BAS-R2)
P.g. non-smokers	1.00	0.04	***P.g.*** non-smokers	0.2	0.1
smokers	0.09	0.02	smokers	0.3	0.5
A.a. non-smokers	0.36	0.33	***A.a.*** non-smokers	0.5	0.2
smokers	0.40	0.53	smokers	0.6	0.3
P.i. non-smokers	0.83	0.14	***P.i.*** non-smokers	0.2	1.0
smokers	0.67	0.35	smokers	0.7	0.9
T.d. non-smokers	0.72	0.18	***T.d.*** non-smokers	0.1	0.3
smokers	0.60	0.10	smokers	0.4	0.6
B.f. non-smokers	0.65	0.59	***B.f.*** non-smokers	0.4	0.6
smokers	0.53	0.06	smokers	0.1	0.1

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 55. Comparison of GCF volume between non-smokers and smokers before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=52 / 28 $N_{FM\text{-}SRP}$=48 / 40	Baseline	p-value[2]	R1	R2	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
Q-SRP non-smokers	0.51 ± 0.31	0.030	0.22 ± 0.20	0.17 ± 0.13	0.28 ± 0.35	<0.001	0.100	0.33 ± 0.32	<0.001	0.010
smokers	0.40 ± 0.37		0.17 ± 0.14	0.21 ± 0.12	0.23 ± 0.34	<00.01		0.19 ± 0.40	0.030	
FM-SRP non-smokers	0.42 ± 0.25	0.200	0.18 ± 0.15	0.22 ± 0.17	0.23 ± 0.24	<0.001	0.370	0.21 ± 0.29	<0.001	0.630
smokers	0.36 ± 0.29		0.15 ± 0.12	0.15 ± 0.10	0.21 ± 0.31	<0.001		0.19 ± 0.32	<0.001	

GCF volume is expressed as µl/30sec

Mean ± sd

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

[1]P-value is given for changes in each subgroup before/after SRP

[2]P-value is given for differences between non-smokers and smokers in each treatment group.

Table 56. GCF antibody titres in non-smokers and smokers before and after Q-SRP.

$N_{non\text{-}smokers}$=52 $N_{smokers}$=28	Baseline	p-value (BAS)	R1	p-value (BAS-R1)	R2	p-value (BAS-R2)
P.g. non-smokers	30.8 ± 22.0	0.010	2951.0 ± 1896.0	0.561	23.0 ± 9.0	0.773
smokers	1.8 ± 0.7		2.0 ± 0.5		4.0 ± 1.9	
A.a. non-smokers	8031.0 ± 5738.0	0.002	187.0 ± 179.0	0.947	17.2 ± 13.1	0.055
smokers	2.0 ± 0.6		1.4 ± 0.4		2.0 ± 0.6	
P.i. non-smokers	3.4 ± 1.0	0.002	2.4 ± 0.4	0.951	2.1 ± 0.3	0.958
smokers	1.4 ± 0.5		1.1 ± 0.2		1.7 ± 0.5	
T.d. non-smokers	5.6 ± 1.9	0.021	0.7 ± 0.2	0.037	1.3 ± 0.4	0.002
smokers	2.1 ± 0.8		1.5 ± 0.6		2.5 ± 0.9	
B.f. non-smokers	2.4 ± 0.9	0.505	2.4 ± 0.9	0.979	2.1 ± 1.1	0.789
smokers	2.6 ± 1.8		1.1 ± 0.2		2.6 ± 1.8	

GCF IgG titres are expressed as EU/30sec

Mean ± SEM

P-values are given for differences between non-smokers and smokers

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

Q-SRP = quadrant scaling and root planing

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*.

Table 57. Percentage of sites positive for the five putative periodontal pathogens in non-smokers and smokers before and after Q-SRP.

$N_{non\text{-}smokers}$=52 $N_{smokers}$=28	Baseline	p-value[2]	R1	p-value[2]	R2	p-value[2]	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
P.g. non-smokers	29.0	0.140	2.0	1.000	0.0		27.0	0.001	0.110	29.0	<0.001	0.110
smokers	46.5		0.0		0.0		46.5	<0.001		46.5	<0.001	
A.a. . non-smokers	21.0	0.980	4.0	0.610	4.0	0.340	17.0	0.022	0.450	17.0	0.022	0.360
smokers	21.0		7.0		11.0		14.0	0.125		11.0	0.250	
P.i. non-smokers	38.5	0.700	11.5	1.000	8.0	0.270	27.0	0.003	0.790	31.0	<0.001	0.700
smokers	43.0		11.0		18.0		32.0	0.012		25.0	0.039	
T.d. non-smokers	46.0	0.370	4.0	1.000	2.0	0.280	44.0	<0.001	0.290	44.0	<0.001	0.170
smokers	36.0		3.6		7.0		32.0	0.004		29.0	0.008	
B.f. non-smokers	81.0	0.340	8.0	0.690	10.0	0.700	75.0	<0.001	0.180	73.0	<0.001	0.140
smokers	71.5		11.0		14.0		61.0	<0.001		57.0	<0.001	

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

[1]P-value is given for changes before/after Q-SRP

[2]P-value is given for differences between non-smokers and smokers.

Table 58. Percentage of patients positive for the five putative periodontal pathogens in non-smokers and smokers before and after Q-SRP.

$N_{non\text{-}smokers}$=13 $N_{smokers}$=7	Baseline	p-value[2]	R1	p-value[2]	R2	p-value[2]	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
P.g. non-smokers	31.0	0.36	8.0	1.00	0.0		23.0	0.38	0.36	31.0	0.13	0.36
smokers	57.0		0.0		0.0		57.0	0.13		57.0	0.13	
A.a. . non-smokers	38.5	1.00	15.0	1.00	15.0	1.00	23.0	0.38	0.61	23.0	0.38	0.61
smokers	28.5		14.0		14.0		14.0	1.0		14.0	1.00	
P.i. non-smokers	69.0	0.65	38.5	1.00	31.0	0.65	31.0	0.28	0.64	38.5	0.13	0.64
smokers	57.0		28.5		43.0		29.0	0.50		14.0	1.00	
T.d. non-smokers	61.5	0.64	15.0	1.00	8.0	1.00	46.0	0.07	0.37	54.0	<0.02	0.37
smokers	43.0		14.0		14.0		28.5	0.50		28.5	0.50	
B.f. non-smokers	92.0	0.99	31.0	0.65	38.5	1.00	61.5	0.02	0.36	54.0	0.04	0.99
smokers	86.0		43.0		29.0		43.0	0.25		57.0	0.13	

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

[1]P-value is given for changes before/after Q-SRP

[2]P-value is given for differences between non-smokers and smokers.

site- and a patient-based analysis. The site-based analysis revealed that there were significant reductions in the prevalence of the bacteria after treatment for each subgroup, except for *A. actinomycetemcomitans* in smokers (Table 57). On a patient-based analysis, the only statistically significant microbiological changes folowing treatment for each subgroup were found in non-smokers for *T. denticola* at R2 ($p<0.05$) and for *B. forsythus* at R1 and R2 ($p<0.05$) (Table 58).

6.1.3 Effect of smoking on clinical, immunological and microbiological parameters in the FM-SRP group

Ten smokers and 12 non-smokers were included in this group and the whole-mouth clinical indices between non-smokers and smokers before and after FM-SRP were analysed (Table 59). No significant differences were seen in any clinical index between non-smokers and smokers before and after treatment indicating that smokers and non-smokers had similar clinical improvement after FM-SRP.

Table 60 shows the site-specific clinical indices and their change with treatment in relation to smoking status for the FM-SRP group. Non-smokers tended to have slightly deeper pockets by 0.3 mm, lower RAL by 0.2 mm and greater MGI than smokers at baseline, but these observations were not statistically significant. No significant differences in BOP, PI and suppuration were seen between non-smokers and smokers at baseline. Following FM-SRP, there was a significantly greater clinical improvement for non-smokers, in terms of PD reduction and RAL gain. Non-smokers had an additional PD reduction of 0.5 mm and 0.8 mm over smokers at R1 ($p<0.05$) and R2 ($p<0.01$) from BAS, respectively. Similarly, an increase of approximately 0.7 mm and 0.8 mm gain in RAL was noted for non-smokers over smokers at R1 ($p<0.005$) and R2 ($p<0.01$) from BAS, respectively. However, when the changes in BOP were examined, smokers had a greater BOP reduction over the non-smokers between BAS and R2, although this failed to reach statistical significance ($p=0.051$). This observation is in contrast with findings in the Q-SRP group. 4.0% of sites that were initially negative for BOP turned positive at R1 for non-smokers. This value was 7.5% for smokers. Nevertheless, non-smokers and smokers had a significant improvement for all clinical parameters following treatment ($p<0.05$).

Table 59. Comparison of whole-mouth clinical indices between smokers and non-smokers before and after FM-SRP.

$N_{non\text{-}smokers}$=12 $N_{smokers}$=10	Baseline	p-value[2]	R1	R2	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
PD(mm) non-smokers	4.3 ± 0.6	0.83	2.7 ± 0.2	2.5 ± 0.2	1.6 ± 0.5	<0.001	0.88	1.7 ± 0.5	<0.001	0.98
smokers	4.4 ± 0.7		2.8 ± 0.3	2.7 ± 0.3	1.6 ± 0.6	<0.001		1.7 ± 0.7	<0.001	
AL(mm) non-smokers	4.8 ± 0.8	0.12	3.6 ± 0.6	3.4 ± 0.5	1.2 ± 0.4	<0.001	0.25	1.2 ± 0.4	<0.001	0.49
smokers	5.5 ± 1.1		4.5 ± 1.2	4.4 ± 1.2	1.0 ± 0.4	<0.001		1.0 ± 0.6	<0.001	
BOP(%) non-smokers	66.0 ± 18.0	0.30	18.0 ± 9.0	12.0 ± 7.0	48.0 ± 17.0	<0.001	0.16	53.0 ± 20.0	<0.001	0.23
smokers	73.0 ± 15.0		16.5 ±11.0	9.5 ± 6.0	57.0 ± 11.0	<0.001		63.0 ± 15.0	<0.001	
No. sites≥5 mm										
non-smokers	64.0 ± 25.0	0.65	12.0 ± 8.0	8.0 ± 6.0	52.0 ± 21.0	<0.001	0.79	48.0 ± 14.0	<0.001	0.31
smokers	69.0 ± 30.0		14.5 ± 5.0	8.6 ± 5.0	55.0 ± 27.0	<0.001		60.0 ± 32.0	<0.001	

Mean ± sd

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

PD = pocket depth; AL = attachment level; BOP = bleeding on probing;

[1]P-value is given for changes before/after FM-SRP

[2]P-value is given for differences between non-smokers and smokers.

Table 60. Comparison of site-specific clinical indices between smokers and non-smokers before and after FM-SRP.

$N_{non\text{-}smokers}$=48 $N_{smokers}$=40		Baseline	p-value[2]	R1	R2	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
PD(mm)	non-smokers	6.0 ± 1.2	0.140	3.7 ± 0.9	3.1 ± 0.8	2.3 ± 1.1	<0.001	0.018	3.0 ± 1.4	<0.001	0.007
	smokers	5.7 ± 0.7		3.9 ± 0.9	3.5 ± 0.8	1.8 ± 0.9	<0.001		2.2 ± 1.0	<0.001	
RAL(mm)	non-smokers	13.8 ± 2.0	0.540	12.8 ± 1.6	12.2 ± 1.7	1.0 ± 0.8	<0.001	<0.005	1.5 ± 1.0	<0.001	<0.006
	smokers	14.0 ± 1.6		13.7 ± 1.7	13.3 ± 1.8	0.3 ± 0.8	0.014		0.7 ± 0.9	<0.001	
BOP*	non-smokers	77.0	0.740	21.0	20.0	56.3	<0.001	0.500	57.5	<0.001	0.051
	smokers	80.0		20.0	3.0	60.0	<0.001		80.5	<0.001	
Sup*	non-smokers	60.4	0.350	2.0	2.5	58.0	<0.001	0.520	57.5	<0.001	0.900
	smokers	70.0		5.0	5.6	65.0	<0.001		61.0	<0.001	
MGI	non-smokers	2.6 ± 0.7	0.200	0.4 ± 0.5	0.3 ± 0.4	2.2 ± 0.8	<0.001	0.150	2.3 ± 0.8	<0.001	0.600
	smokers	2.4 ± 0.6		0.6 ± 0.5	0.3 ± 0.5	1.8 ± 0.7	<0.001		2.1 ± 0.8	<0.001	
PI	non-smokers	2.0 ± 0.7	0.340	0.8 ± 0.7	0.7 ± 0.6	1.3 ± 0.8	<0.001	0.400	1.5 ± 0.7	<0.001	0.300
	smokers	1.9 ± 0.7		0.7 ± 0.6	0.5 ± 0.7	1.3 ± 1.0	<0.001		1.4 ± 1.1	<0.001	

Mean ± sd; *% of positive sites

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

PD = pocket depth; RAL = relative attachment level; BOP = bleeding on probing; Sup = suppuration; MGI = modified gingival index; PI = plaque index

[1]P-value is given for changes before/after FM-SRP; [2]p-value is given for differences between non-smokers and smokers.

The analyses of serum IgG titres (EU) showed that there were no statistically significant differences between non-smokers and smokers before and after FM-SRP (Table 61). Table 62 summarises the statistical significance of the changes in serum IgG titres after FM-SRP for each subgroup. For non-smokers, there was a significant decrease in IgG levels to *T. denticola* between BAS and R1 ($p<0.01$), and for smokers antibody titres to *A. actinomycetemcomitans* and *P. intermedia* significantly decreased at this time point ($p<0.05$).

No significant differences in IgG avidity (M at ID_{50}) were detected between the two subgroups at BAS or at R1 and R2 (Table 63). Nevertheless, when the changes with treatment were examined, a greater increase in antibody avidity against *P. gingivalis* was found for smokers compared to non-smokers between BAS-R1 and BAS-R2 ($p<0.05$). Table 62 summarises the levels of significance for the changes in IgG avidity in each subgroup following FM-SRP. For non-smokers, no statistically significant increase in IgG avidity was found after treatment. Antibody avidity to *P. gingivalis* significantly increased between BAS and R1 for smokers ($p<0.05$).

Table 55 (section 6.1.2) compares the GCF volume (µl / 30 sec) between non-smokers and smokers before and after FM-SRP. No significant differences were found between the two subgroups at baseline and after treatment. Both subgroups had a significant reduction in GCF volume following FM-SRP ($p<0.01$). At baseline, GCF antibody titres (EU / 30 sec) were found to be significantly lower in smokers than non-smokers for *A. actinomycetemcomitans* ($p<0.05$) and *P. intermedia* ($p<0.05$) (Table 64). The post-treatment changes revealed that non-smokers had a significantly greater reduction in GCF antibody titres to *P. gingivalis* between BAS and R2 ($p<0.05$) and to *T. denticola* between BAS and R1 ($p<0.05$).

The prevalence in subgingival plaque of the five suspected periodontal pathogens between non-smokers and smokers before and after FM-SRP was analysed and the results showed no significant differences for any pathogen on a patient-based analysis (Table 65). The comparison of the percentage of patients positive for the bacteria before and after SRP for each subgroup revealed statistically significant reductions after treatment only for *T. denticola* and *B. forsythus* in non-smokers ($p<0.01$) and smokers ($p<0.05$). Table 66 shows that on a site-based analysis a significantly higher proportion

Table 61. Serum IgG titres in smokers and non-smokers before and after FM-SRP.

$N_{non\text{-}smokers}$ =12 $N_{smokers}$ =10	Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g. non-smokers	88.0 (48.0, 473.0)	0.3	100.0 (52.0, 255.0)	0.5	137.0 (25.0, 173.0)	0.9	22.0 (-31.0, 356.0)	0.2	34.0 (-38.0, 8405.0)	0.4
smokers	50.0 (19.0, 915.0)		54.0 (14.0, 1432.0)		55.0 (27.0, 1094.0)		-4.0 (-339.0, 12.0)		4.0 (-83.0, 946.0)	
A.a. non-smokers	57.0 (14.0, 2161.0)	0.5	34.0 (17.0, 1509.0)	0.5	64.0 (18.0, 4008.0)	0.5	2.0 (-7.0, 36.0)	0.3	22.0 (3.0, 4618.0)	0.5
smokers	52.0 (10.0, 774.0)		38.0 (11.0, 146.0)		51.0 (13.0, 194.0)		13.5 (-0.3, 335.0)		4.0 (-5.0, 686.0)	
P.i. non-smokers	325.0 (185.0, 746.0)	0.6	292.0 (104.0, 524.0)	0.4	347.0 (225.0, 557.0)	0.6	45.0 (-20.0, 231.0)	0.5	-6.0 (-47.0, 206.0)	0.4
smokers	252.0 (42.0, 908.0)		185.0 (46.0, 481.0)		191.0 (108.0, 532.0)		68.0 (4.0, 282.0)		63.0 (-41.0, 491.0)	
T.d. non-smokers	13.0 (7.0, 60.0)	0.9	8.5 (5.0, 28.0)	0.7	14.0 (6.0, 27.0)	0.7	5.5 (1.0, 36.0)	0.8	4.5 (1.5, 68.0)	0.7
smokers	21.5 (7.0, 75.0)		14.0 (6.0, 27.0)		15.0 (12.0, 24.0)		11.0 (-1.3, 44.0)		9.0 (-0.5, 74.0)	
B.f. non-smokers	32.0 (13.0, 676.0)	0.8	0.4 (0.3, 0.6)	0.3	0.5 (0.4, 0.8)	0.8	7.0 (-1.0, 84.0)	0.5	6.0 (-5.0, 183.0)	0.9
smokers	33.0 (13.0, 1753.0)		0.6 (0.4, 0.7)		0.4 (0.3, 1.0)		1.0 (-13.0, 119.0)		1.0 (-5.0, 820.0)	

Serum IgG titres are expressed as ELISA units

Median (Q1, Q3)

P-values are given for differences between non-smokers and smokers

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 62. P-values for changes in serum IgG titres and IgG avidity in non-smokers and smokers after FM-SRP.

IgG titres	p-value (BAS-R1)	p-value (BAS-R2)	IgG avidity	p-value (BAS-R1)	p-value (BAS-R2)
P.g. non-smokers	0.260	0.180	***P.g.*** non-smokers	0.150	0.500
smokers	0.520	0.720	smokers	<0.030	0.100
A.a. non-smokers	0.780	0.090	***A.a.*** non-smokers	0.080	0.100
smokers	0.028	0.240	smokers	0.600	0.080
P.i. non-smokers	0.078	0.760	***P.i.*** non-smokers	0.300	1.000
smokers	0.028	0.120	smokers	0.290	0.200
T.d. non-smokers	0.006	0.060	***T.d.*** non-smokers	0.080	0.200
smokers	0.074	0.070	smokers	0.540	0.600
B.f. non-smokers	0.160	0.220	***B.f.*** non-smokers	0.410	0.100
smokers	0.680	0.310	smokers	0.440	0.600

Median (Q1, Q3)

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 63. IgG avidity in smokers and non-smokers before and after FM-SRP.

$N_{non\text{-}smokers}$=12 $N_{smokers}$=10	Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g. non-smokers	0.52 (0.30, 0.70)	0.37	0.45 (0.30, 0.70)	0.50	0.65 (0.30, 0.80)	0.97	0.11 (-0.05, 0.20)	0.04	0.05 (-0.09, 0.20)	0.04
smokers	0.47 (0.30, 0.60)		0.59 (0.50, 0.70)		0.54 (0.40, 1.10)		-0.11 (-0.20, -0.03)		-0.06 (-0.60, -0.02)	
A.a. non-smokers	0.48 (0.40, 0.70)	0.62	0.47 (0.40, 1.20)	0.19	0.55 (0.40, 0.90)	0.54	-0.08 (-0.50, 0.04)	0.14	-0.12 (-0.40, 0.06)	1.00
smokers	0.43 (0.30, 0.70)		0.42 (0.40, 0.60)		0.55 (0.30, 0.80)		0.02 (-0.04, 0.05)		-0.09 (0.30, 0.02)	
P.i. non-smokers	0.88 (0.60, 1.20)	0.97	0.94 (0.60, 1.30)	0.53	0.87 (0.70, 1.60)	0.68	-0.02 (-0.10, 0.02)	0.11	0.04 (-0.20, 0.10)	0.27
smokers	0.84 (0.70, 1.10)		0.82 (0.70, 1.00)		0.76 (0.70, 1.20)		0.09 (-0.04, 0.20)		0.09 (0.00, 0.20)	
T.d. non-smokers	0.33 (0.30, 0.50)	0.53	0.44 (0.30, 0.90)	1.00	0.38 (0.30, 0.50)	0.54	-0.10 (-0.70, 0.05)	0.47	-0.03 (-0.14, 0.03)	0.24
smokers	0.43 (0.20, 0.60)		0.51 (0.30, 0.80)		0.40 (0.40, 0.50)		-0.05 (-0.30, 0.10)		0.03 (-0.13, 0.20)	
B.f. non-smokers	0.46 (0.30, 0.60)	0.64	0.41 (0.30, 0.60)	0.25	0.54 (0.40, 0.80)	0.77	0.02 (-0.06, 0.10)	0.47	-0.08 (-0.20, 0.05)	0.81
smokers	0.47 (0.30, 0.60)		0.55 (0.40, 0.70)		0.40 (0.30, 1.00)		0.02 (0.30, 0.09)		0.02 (-0.40, 0.06)	

IgG avidity is expressed as molarity (M) at ID_{50}

Median (Q1, Q3)

P-values are given for differences between non-smokers and smokers

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 64. GCF antibody titres in non-smokers and smokers before and after FM-SRP.

$N_{non\text{-}smokers}$=48 $N_{smokers}$=40	Baseline	p-value (BAS)	R1	p-value (BAS-R1)	R2	p-value (BAS-R2)
P.g. non-smokers	26.2 ± 20.0	0.870	4.0 ± 0.7	0.304	22.6 ± 20.0	0.014
smokers	2.2 ± 0.4		3.8 ± 1.0		6.2 ± 2.4	
A.a. non-smokers	630.0 ± 247.0	0.045	2810.0 ± 2006.0	0.240	308.0 ± 180.0	0.450
smokers	4.6 ± 2.5		4.3 ± 2.4		7.8 ± 4.8	
P.i. non-smokers	6.4 ± 3.9	0.042	2.4 ± 0.3	0.151	3.0 ± 0.4	0.883
smokers	1.8 ± 0.6		2.5 ± 0.5		2.1 ± 0.2	
T.d. non-smokers	11.4 ± 5.5	0.976	3.0 ± 0.6	0.034	2.5 ± 0.9	0.991
smokers	4.1 ± 1.0		1.1 ± 0.3		2.1 ± 1.1	
B.f. non-smokers	2.2 ± 1.2	0.302	3.6 ± 1.4	0.201	1.1 ± 0.2	0.052
smokers	0.7 ± 0.1		0.9 ± 0.1		1.2 ± 0.1	

GCF IgG titres are expressed as EU/30sec

Mean ± SEM

P-values are given for differences between non-smokers and smokers

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

FM-SRP = full mouth scaling and root planing

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus.*

Table 65. Percentage of patients positive for the five putative periodontal pathogens in non-smokers and smokers before and after FM-SRP.

$N_{non\text{-}smokers}$=12 $N_{smokers}$=10	Baseline	p-value[2]	R1	p-value[2]	R2	p-value[2]	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
P.g. non-smokers	42.0	0.700	8.0	1.000	0	0.500	33.0	0.125	1.000	40.0	0.125	0.600
smokers	30.0		0		11.0		30.0	0.250		22.0	0.500	
A.a. . non-smokers	42.0	0.400	25.0	0.200	10.0	1.000	17.0	0.500	1.000	40.0	0.125	0.600
smokers	20.0		0		0		20.0	0.500		22.0	0.500	
P.i. non-smokers	67.0	0.400	8.0	1.000	40.0	0.300	58.0	0.160	0.700	30.0	0.250	1.000
smokers	40.0		0		11.0		40.0	0.125		33.0	0.250	
T.d. non-smokers	92.0	1.000	0		10.0	1.000	92.0	0.001	1.000	80.0	0.008	1.000
smokers	100.0		0		11.0		100.0	0.002		89.0	0.008	
B.f. non-smokers	100.0	0.500	8.0	0.600	20.0	0.600	92.0	0.001	0.300	80.0	0.008	0.600
smokers	90.0		20.0		33.0		70.0	0.016		56.0	0.125	

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

[1]P-value is given for changes before/after FM-SRP

[2]P-value is given for differences between non-smokers and smokers.

Table 66. Percentage of sites positive for the five putative periodontal pathogens in non-smokers and smokers before and after FM-SRP.

$N_{non\text{-}smokers}$=45 $N_{smokers}$=34	Baseline	p-value[2]	R1	p-value[2]	R2	p-value[2]	Change (BAS-R1)	p-value[1]	p-value[2]	Change (BAS-R2)	p-value[1]	p-value[2]
P.g. non-smokers	38.0	0.990	4.0	0.500	0.0	0.470	33.0	<0.001	0.210	35.0	<0.001	0.29
smokers	21.0		0.0		3.0		21.0	0.016		23.0	0.016	
A.a. non-smokers	33.0	0.026	19.0	0.003	2.5	0.990	13.0	0.146	0.330	38.0	<0.001	0.02
smokers	12.0		0.0		0.0		12.0	0.125		13.0	0.125	
P.i. non-smokers	47.0	0.035	2.0	1.000	12.5	0.200	44.0	0.001	0.054	32.5	0.002	0.46
smokers	24.0		0.0		3.0		24.0	0.008		23.0	0.039	
T.d. non-smokers	42.0	0.340	0.0		2.5	0.600	42.0	<0.001	0.340	46.0	<0.001	0.95
smokers	53.0		0.0		5.5		53.0	<0.001		47.0	<0.001	
B.f. non-smokers	84.5	0.800	2.0	0.320	5.0	0.400	82.0	<0.001	0.350	78.0	<0.001	0.40
smokers	82.4		7.5		11.0		73.5	<0.001		70.0	<0.001	

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

[1]P-value is given for changes before/after FM-SRP

[2]P-value is given for differences between non-smokers and smokers.

of sites of non-smokers harboured *A. actinomycetemcomitans* at baseline ($p<0.05$) and at R1 ($p<0.005$) and a greater reduction of this pathogen was seen for non-smokers than smokers at R2 from baseline ($p<0.05$). For *P. intermedia*, a significantly higher percentage of sites of non-smokers was positive for this pathogen at baseline ($p<0.05$). When each subgroup was analysed on a site-basis for changes following FM-SRP, statistically significant reductions were noted for all pathogens ($p<0.05$), except for *A. actinomycetemcomitans* in sites of smokers.

6.1.4 Comparison of smokers before and after Q-SRP and FM-SRP

To investigate whether the differences in clinical and immunological parameters between smokers in Q-SRP and FM-SRP groups were the result of therapy or due to differences in the frequency of cigarette usage within smokers, a comparison of the periodontal parameters was made between smokers in the Q-SRP and FM-SRP groups.

Table 67, shows the differences of the whole-mouth clinical indices between smokers in Q-SRP group (n=7) and in FM-SRP group (n=10). No differences in any clinical index were detected between therapy groups at baseline. The changes in PD, AL and No. of sites $\geq$ 5 mm with treatment were similar for both groups. However, a significantly greater BOP reduction was noted for smokers in the FM-SRP group between BAS and R2 ($p<0.05$).

Tables 50 and 51 (section 6.1.2) and Tables 59 and 60 (section 6.1.3) depict the changes in the whole-mouth and the site-specific clinical indices for Q-SRP and FM-SRP groups, respectively, based on smoking status. The GLM analysis demonstrated that there was a significant three-way interaction of smoking and treatment approach (Q-SRP and FM-SRP) and visit effect on PD and RAL of the selected sites. This finding is difficult to assess, but it seems that smoking is the predominant factor in this three-way interaction. It is apparent from these Tables that Q-SRP non-smokers showed the greater PD reduction between BAS and R1 compared to Q-SRP smokers and FM-SRP non-smokers and smokers. The analysis of the site-specific clinical indices between smokers in the Q-SRP group and smokers in the FM-SRP group showed that there were no significant differences in site-specific clinical indices between the two subgroups at baseline, despite the greater BOP and Sup seen for FM-SRP smokers (Table 68). The analysis of the changes in local clinical parameters with treatment showed that FM-SRP

Table 67. Comparison of whole-mouth clinical indices between Q-SRP smokers and FM-SRP smokers.

$N_{Q\text{-}SRP}=7$ $N_{FM\text{-}SRP}=10$		Baseline	p-value	R1	R2	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
PD(mm)	Q-SRP	4.3 ± 0.8	0.840	2.8 ± 0.4	2.8 ± 0.3	1.5 ± 0.5	0.840	1.5 ± 0.5	0.780
	FM-SRP	4.4 ± 0.7		2.8 ± 0.3	2.7 ± 0.3	1.6 ± 0.6		1.7 ± 0.7	
AL(mm)	Q-SRP	4.9 ± 1.0	0.280	3.8 ± 1.0	3.8 ± 1.0	1.1 ± 0.5	0.730	1.1 ± 0.5	0.870
	FM-SRP	5.5 ± 1.1		4.5 ± 1.2	4.4 ± 1.2	1.0 ± 0.4		1.1 ± 0.6	
BOP(%)	Q-SRP	63.0 ± 17.0	0.260	19.0 ± 10.0	17.0 ± 8.0	44.0 ± 12.0	0.053	46.0 ± 14.0	0.040
	FM-SRP	73.0 ± 15.0		16.5 ± 11.0	9.5 ± 6.0	56.5 ± 11.0		63.5 ± 15.0	
No. sites≥5 mm									
	Q-SRP	64.0 ± 25.0	0.710	14.0 ± 17.0	10.0 ± 11.0	50.0 ± 20.0	0.680	54.0 ± 20.0	0.670
	FM-SRP	69.0 ± 30.0		14.5 ± 5.0	9.0 ± 5.0	54.5 ± 27.0		60.0 ± 31.0	

Mean ± sd

Q-SRP = quadrant scaling and root planing ; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

PD = pocket depth; AL = attachment level; BOP = bleeding on probing

P-values are given for differences between Q-SRP smokers and FM-SRP smokers.

Table 68. Comparison of site-specific clinical indices between Q-SRP smokers and FM-SRP smokers.

$N_{Q\text{-}SRP}$=28 $N_{FM\text{-}SRP}$=40		Baseline	p-value	R1	R2	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
PD(mm)	Q-SRP	5.9 ± 1.0	0.26	4.1 ± 1.2	3.7 ± 0.7	1.9 ± 1.1	0.77	2.2 ± 0.9	0.91
	FM-SRP	5.7 ± 0.7		3.9 ± 0.9	3.5 ± 0.8	1.8 ± 0.9		2.2 ± 1.0	
RAL(mm)	Q-SRP	14.4 ± 2.0	0.33	14.0 ± 2.0	13.8 ± 2.0	0.38 ± 0.9	0.83	0.6 ± 0.9	0.65
	FM-SRP	14.0 ± 1.6		13.7 ± 1.7	13.3 ± 1.8	0.33 ± 0.8		0.7 ± 0.9	
BOP*	Q-SRP	64.0	0.15	18.0	28.6	46.5	0.082	36.0	0.01
	FM-SRP	80.0		20.0	3.0	60.0		80.5	
Sup*	Q-SRP	57.0	0.28	3.6	0.0	54.0	0.34	57.0	0.75
	FM-SRP	70.0		5.0	5.5	65.0		61.0	
MGI	Q-SRP	2.2 ± 0.5	0.16	0.4 ± 0.6	0.3 ± 0.4	1.8 ± 0.7	0.17	1.9 ± 0.5	0.63
	FM-SRP	2.4 ± 0.6		0.6 ± 0.5	0.3 ± 0.5	1.8 ± 0.7		2.1 ± 0.8	
PI	Q-SRP	2.0 ± 0.7	0.70	0.8 ± 0.8	0.6 ± 0.6	1.2 ± 0.7	0.77	1.4 ±0.7	0.66
	FM-SRP	1.9 ± 0.7		0.7 ± 0.6	0.5 ± 0.7	1.3 ± 1.0		1.4 ± 1.1	

Mean ± sd; *% of positive sites

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

PD = pocket depth; RAL = relative attachment level; BOP = bleeding on probing; Sup = suppuration; MGI = modified gingival index; PI = plaque index

P-values compare sites of Q-SRP smokers and FM-SRP smokers.

resulted in significantly greater BOP reduction than Q-SRP between BAS and R2 ($p=0.01$).

The immunological data were analysed and no differences in serum IgG titres (EU) against any bacteria were found between the two subgroups at baseline (Table 69). However, at R2 significantly higher serum antibody levels were detected against *P. intermedia* for FM-SRP smokers than Q-SRP smokers ($p<0.05$). In addition, between BAS and R1 there was a significantly greater reduction in serum antibody levels to this pathogen for FM-SRP smokers compared to Q-SRP smokers ($p<0.05$).

Table 70 compares the IgG avidity (M at ID_{50}) in smokers before and after Q-SRP and FM-SRP. At baseline, IgG avidity to *B. forsythus* was significantly higher for FM-SRP smokers than Q-SRP smokers ($p<0.05$), but there was no significant difference between the two groups at R1 and R2. FM-SRP smokers showed a significantly greater increase in antibody avidity to *P. gingivalis* between BAS and R1 than did Q-SRP smokers ($p<0.05$).

Table 71 compares the differences of GCF volume (μl / 30 sec) between smokers in Q-SRP and smokers in FM-SRP groups. No significant differences in GCF volume were found between smokers of the two treatment groups before and after treatment. The comparison of GCF IgG titres (EU / 30 sec) between smokers of Q-SRP and FM-SRP groups demonstrated a significantly greater reduction in GCF IgG titres to *T. denticola* between BAS and R2 for FM-SRP smokers than Q-SRP smokers ($p=0.01$) (Table 72).

The prevalence of the putative periodontal pathogens at sites of smokers between the Q-SRP and FM-SRP groups is depicted in Table 73, and it shows a significantly higher prevalence of *P. gingivalis* for the Q-SRP group at baseline ($p<0.05$). At R1, *P. gingivalis* was not detected at the selected sites of smokers in both groups, but between BAS and R1 the decrease of this pathogen was greater for Q-SRP smokers than FM-SRP smokers ($p<0.05$). No significant differences in the prevalence of the other bacteria were noted before and after SRP. Table 74 shows the patient-based analysis of the PCR data between smokers in the two treatment groups. Smokers in the FM-SRP group showed significantly a higher prevalence of *T. denticola* at baseline compared to Q-SRP group ($p<0.05$) and a significantly greater reduction of this pathogen between

Table 69. Comparison of serum IgG titres between Q-SRP smokers and FM-SRP smokers.

$N_{Q\text{-}SRP}$=7 $N_{FM\text{-}SRP}$=10		Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g.	Q-SRP	70.0 (9.0, 516.0)	0.92	76.0 (5.0. 255.0)	0.80	39.0 (8.0, 228.0)	0.34	42.0 (0.0, 261.0)	0.07	31.0 (1.0, 391.0)	0.29
	FM-SRP	50.0 (19.0, 915.0)		54.0 (14.0, 1432.0)		55.0 (27.0, 1094.0)		-4.0 (-339.0, 12.0)		4.0 (-83.0, 946.0)	
A.a.	Q-SRP	21.0 (5.0, 67.0)	0.35	12.0 (5.0, 58.0)	0.32	21.0 (4.0, 25.0)	0.15	2.0 (0.0, 11.0)	0.35	2.0 (-5.0, 34.0)	0.67
	FM-SRP	52.0 (10.0, 774.0)		38.0 (11.0, 146.0)		51.0 (13.0, 194.0)		13.5 (-0.3, 335.0)		4.0 (-5.0, 686.0)	
P.i.	Q-SRP	60.0 (20.0, 162.0)	0.24	43.0 (24.0, 173.0)	0.40	42.0 (24.0, 129.0)	0.03	2.0 (-39.0, 6.0)	0.02	6.0 (-16.0, 70.0)	0.50
	FM-SRP	252.0 (42.0, 908.0)		185.0 (46.0, 481.0)		191.0 (108.0, 532.0)		68.0 (4.0, 282.0)		63.0 (-41.0, 491.0)	
T.d.	Q-SRP	4.0 (1.0, 14.0)	0.15	3.0 (2.0, 19.0)	0.24	1.0 (1.0, 14.0)	0.06	-1.0 (-5.0, 2.0)	0.09	2.0 (0.0, 3.0)	0.34
	FM-SRP	21.5 (7.0, 75.0)		14.0 (6.0, 27.0)		15.0 (12.0, 24.0)		11.0 (-1.3.0, 44.0)		9.0 (-0.5, 74.0)	
B.f.	Q-SRP	28.0 (12.0, 117.0)	0.96	0.4 (0.3, 0.5)	0.19	0.4 (0.2, 1.1)	0.52	1.0 (-6.0, 88.0)	0.77	19.0 (0.0, 63.0)	0.56
	FM-SRP	33.0 (13.0, 1753.0)		0.6 (0.4, 0.7)		0.4 (0.3, 1.0)		1.0 (-13.0, 119.0)		1.0 (-5.0, 820.0)	

Serum IgG titres are expressed as ELISA units (EU)

Median (Q1, Q3)

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

P-values are given for differences between Q-SRP smokers and FM-SRP smokers.

Table 70. Comparison of IgG avidity between Q-SRP smokers and FM-SRP smokers.

$N_{Q\text{-}SRP}=7$ $N_{FM\text{-}SRP}=10$		Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g.	Q-SRP	0.41 (0.30, 0.60)	0.700	0.40 (0.30, 0.50)	0.055	0.39 (0.30, 1.30)	0.600	0.04 (-0.03, 0.13)	0.030	-0.04 (-0.90, 0.17)	0.670
	FM-SRP	0.47 (0.30, 0.60)		0.59 (0.50, 0.70)		0.54 (0.40, 1.10)		-0.11 (-0.20 -0.03)		-0.06 (-0.60, -0.02)	
A.a.	Q-SRP	0.34 (0.30, 0.50)	0.350	0.33 (0.20, 0.50)	0.240	0.41 (0.30, 0.70)	0.750	0.03 (-0.08, 0.10)	0.690	-0.08 (-0.30, 0.07)	0.670
	FM-SRP	0.43 (0.30, 0.70)		0.42 (0.40, 0.60)		0.55 (0.30, 0.80)		0.02 (-0.04, 0.05)		-0.09 (0.30, 0.02)	
P.i	Q-SRP	0.91 (0.50, 1.30)	0.960	0.84 (0.50, 1.20)	0.690	1.03 (0.70, 1.20)	0.870	0.09 (-0.14, 0.40)	1.000	0.03 (-0.24, 0.20)	0.370
	FM-SRP	0.84 (0.70, 1.10)		0.82 (0.70, 1.00)		0.76 (0.70, 1.20)		0.09 (-0.04, 0.20)		0.09 (0.00, 0.20)	
T.d.	Q-SRP	0.30 (0.20, 0.40)	0.220	0.28 (0.20, 0.70)	0.200	0.25 (0.20, 0.40)	0.100	-0.07 (-0.40, 0.06)	0.800	-0.03 (-0.05, 0.00)	0.520
	FM-SRP	0.43 (0.20, 0.60)		0.51 (0.30, 0.80)		0.40 (0.40, 0.50)		-0.05 (-0.30, 0.10)		0.03 (-0.13, 0.20)	
B.f.	Q-SRP	0.29 (0.20, 0.50)	0.030	0.43 (0.30, 0.50)	0.180	0.38 (0.20, 1.10)	0.520	-0.12 (-0.20, 0.00)	0.430	-0.07 (-0.80, 0.01)	0.300
	FM-SRP	0.47 (0.30, 0.60)		0.55 (0.40, 0.70)		0.40 (0.30, 1.00)		0.02 (0.30, 0.09)		0.02 (-0.40, 0.06)	

IgG avidity is expressed as molarity (M) at ID_{50}

Median (Q1, Q3)

P.g.: P. gingivalis; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

P-values are given for differences between Q-SRP smokers and FM-SRP smokers.

Table 71. GCF volume in smokers before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=28 $N_{FM\text{-}SRP}$=40	Baseline	p-value	R1	R2	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
Q-SRP smokers	0.40 ± 0.37	0.97	0.17 ± 0.14	0.21 ± 0.12	0.23 ± 0.34	0.88	0.19 ± 0.40	0.57
FM-SRP smokers	0.36 ± 0.29		0.15 ± 0.12	0.15 ± 0.10	0.21 ± 0.31		0.19 ± 0.32	

GCF volume is expressed as µl/30sec

Mean ± sd

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

P-values compare sites of Q-SRP smokers and FM-SRP smokers.

Table 72. GCF IgG titres in smokers before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=28 $N_{FM\text{-}SRP}$=40	Baseline	p-value (BAS)	R1	p-value (BAS-R1)	R2	p-value (BAS-R2)
P.g. Q-SRP	1.8 ± 0.7	0.07	2.0 ± 0.5	0.96	3.9 ± 1.9	0.41
FM-SRP	2.2 ± 0.4		3.8 ± 1.0		6.2 ± 2.4	
A.a. Q-SRP	2.0 ± 0.6	0.28	1.4 ± 0.4	0.33	2.0 ± 0.6	0.94
FM-SRP	4.6 ± 2.5		4.3 ± 2.4		7.8 ± 4.8	
P.i. Q-SRP	1.4 ± 0.5	0.12	1.1 ± 0.2	0.17	1.7 ± 0.5	0.26
FM-SRP	1.8 ± 0.6		2.5 ± 0.5		2.1 ± 0.2	
T.d. Q-SRP	2.1 ± 0.8	0.05	1.5 ± 0.6	0.06	2.5 ± 0.9	0.01
FM-SRP	4.1 ± 1.0		1.1 ± 0.3		2.1 ± 1.1	
B.f. Q-SRP	2.6 ± 1.8	0.52	1.1 ± 1.9	0.99	2.6 ± 1.8	0.06
FM-SRP	0.7 ± 0.1		0.9 ± 0.1		1.2 ± 0.1	

GCF IgG titres are expressed as EU/30sec

Mean ± SEM

P-values compare sites of Q-SRP smokers and FM-SRP smokers

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*.

Table 73. Percentage of sites positive for the five putative periodontal pathogens in smokers before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}$=28 $N_{FM\text{-}SRP}$=34		Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g.	Q-SRP	46.5	0.03	0.0		0.0	1.00	46.5	0.03	46.5	0.06
	FM-SRP	21.0		0.0		3.0		21.0		23.0	
A.a.	Q-SRP	21.0	0.30	7.0	0.17	11.0	0.08	14.0	1.00	11.0	1.00
	FM-SRP	12.0		0.0		0.0		12.0		13.0	
P.i	Q-SRP	43.0	0.10	11.0	0.07	18.0	0.08	32.0	0.29	25.0	0.87
	FM-SRP	23.5		0.0		3.0		24.0		23.0	
T.d.	Q-SRP	36.0	0.17	3.6	0.40	7.0	1.00	32.0	0.10	29.0	0.15
	FM-SRP	53.0		0.0		5.5		53.0		47.0	
B.f.	Q-SRP	71.5	0.30	11.0	0.68	14.0	0.70	61.0	0.28	57.0	0.30
	FM-SRP	82.0		7.5		11.0		73.5		70.0	

P-values compare sites of Q-SRP smokers and FM-SRP smokers

P.g.: *P. gingivalis*; *A.a.*: *A. .actinomycetemcomitans*; *P.i.*: *P. intermedia*; *T.d.*: *T. denticola*; *B.f.*: *B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

Table 74. Percentage of patients positive for the five putative periodontal pathogens in smokers before and after Q-SRP and FM-SRP.

$N_{Q\text{-}SRP}=7$ $N_{FM\text{-}SRP}=10$		Baseline	p-value	R1	p-value	R2	p-value	Change (BAS-R1)	p-value	Change (BAS-R2)	p-value
P.g.	Q-SRP	57.0	0.350	0.0		0.0	1.000	57.0	0.350	57.0	0.300
	FM-SRP	30.0		0.0		11.0		30.0		22.0	
A.a.	Q-SRP	28.6	1.000	14.0	0.400	14.0	0.440	14.0	1.000	14.0	1.000
	FM-SRP	20.0		0.0		0.0		20.0		22.0	
P.i	Q-SRP	57.0	0.640	28.6	0.200	43.0	0.260	29.0	1.000	14.0	1.000
	FM-SRP	40.0		0.0		11.0		40.0		33.0	
T.d.	Q-SRP	43.0	<0.020	14.0	0.400	14.0	1.000	28.5	0.003	28.5	<0.040
	FM-SRP	100.0		0.0		11.0		100.0		89.0	
B.f.	Q-SRP	86.0	1.000	43.0	0.600	29.0	1.000	43.0	0.350	57.0	1.000
	FM-SRP	90.0		20.0		33.0		70.0		56.0	

P-values are given for differences between Q-SRP smokers and FM-SRP smokers

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

Q-SRP = quadrant scaling and root planing; FM-SRP = full mouth scaling and root planing

BAS = baseline; R1 = reassessment 1; R2 = reassessment 2.

BAS and R1 ($p<0.005$), and BAS and R2 ($p<0.05$). No significant differences in the prevalence of the other bacteria were found before and after treatment on a subject-based analysis.

6.2 Discussion

6.2.1 Comparison of baseline clinical, immunological and microbiological parameters between non-smokers and smokers

At a site-specific level, smokers showed significantly lower PD, MGI and GCF volume and a trend towards lower bleeding scores at baseline compared to sites of non-smokers. The lower GCF volume found in smokers confirms the results of previous investigations (Holmes, 1990; Kinane and Radvar, 1997). On the other hand, the present data disagree with the findings of other studies that showed deeper pockets in smokers than non-smokers (Bergström and Eliasson, 1987b; Kamma et al. 1999; Haffajee and Socransky, 2001), but it should be noted that the sample size in the current study was smaller than in the quoted studies.

No differences in plaque levels were found between smokers and non-smokers, which is consistent with other reports (Kinane and Radvar, 1997; Kamma et al. 1999; Darby et al. 2000; Haffajee and Socransky, 2001). Therefore, the harmful effects of smoking on periodontal health may not be associated with plaque accumulation and poor oral hygiene (Bergström and Eliasson, 1987a, 1987b). The finding that smoking suppresses the inflammatory response to plaque challenge, and therefore masks clinical signs of gingival inflammation, confirms previous observations in periodontitis patients (Preber and Bergström, 1985; Darby et al. 2000; Bergström and Boström, 2001) and in experimental gingivitis-induced patients (Lie et al. 1998a; Danielsen et al. 1990). However, Bergström et al. (2000) showed that in individuals free of periodontal disease the association between smoking and gingival bleeding was weak.

The present data demonstrated that there were no statistically significant differences in the prevalence of organisms between the two subgroups of patients, on either a site- or a subject-basis. This finding is in agreement with other studies that failed to show an altered subgingival microbiota in smokers (Stoltenberg et al. 1993; Darby et al. 2000; Boström et al. 2001). Lie et al. (1998a) did not attribute the lower bleeding scores seen in smokers after the induction of experimental gingivitis to microbiological differences

between smokers and non-smokers. Nevertheless, there are conflicting data that smokers harbour specific pathogens at higher levels and frequencies than non-smokers (Zambon et al. 1996; Kamma et al. 1999), and that they are at increased risk for bacterial infection (Zambon et al. 1996; Umeda et al. 1998). Disagreement in the subgingival microbial composition between smokers and non-smokers among various studies could be explained by differences in patient populations, in microbial sampling techniques and in detection methods of putative pathogens.

There is evidence that smoking has a systemic effect on the immune response (Andersen et al. 1982; Holt, 1987; Kenney et al. 1977). Present data showed lower serum antibody titres against all microorganisms in smokers than non-smokers, although this observation did not reach statistical significance. This pattern of decreased levels of antibodies was less clear when antibody avidities were considered, showing small differences between the two subgroups of patients. However, GCF antibody titres were lower in smokers than non-smokers and this observation reached significance for some of the tested organisms. Therefore, it appears that smoking can modify the host response and this agrees with previous observations (Haber, 1994). It has been shown in other studies that smoking has an impact on serum immunoglobulin levels, but this effect seems to be both race and serum IgG subclass specific (Quinn et al. 1996; Gunsolley et al. 1997; Tangada et al. 1997; Quinn et al. 1998).

6.2.2 Effect of smoking on clinical, immunological and microbiological parameters in the Q-SRP and FM-SRP groups

At baseline, the whole-mouth clinical indices were similar between smokers and non-smokers in the Q-SRP group. However, on a site-specific basis smokers in the Q-SRP group had less BOP, MGI and GCF volume at baseline confirming previous findings (Preber and Bergström, 1985; Holmes, 1990; Kinane and Radvar, 1997; Darby et al. 2000; Bergström and Boström, 2001). In agreement with previous observations (Haber, 1994), the current study showed that smokers in the Q-SRP group had lower levels of serum IgG antibodies to all tested organisms ($p \leq 0.05$ for *A. actinomycetemcomitans* and *P. intermedia*) and a tendency for lower antibody avidities to most of them. GCF volume was found to be significantly lower for smokers compared to non-smokers and GCF antibody titres against the majority of the organisms were significantly lower for smokers. No significant differences in the prevalence of specific pathogens were seen

on a site- and a subject-basis (Stoltenberg et al. 1993; Darby et al. 2000; Boström et al. 2001). These findings are discussed in the previous section and highlight the notion that smokers have a suppressed inflammatory response and an altered host response to antigenic challenge. In contrast, the subgingival microflora of smokers appears similar to that of non-smokers.

The analysis of the whole-mouth clinical indices showed that following Q-SRP, smokers exhibited significantly less reduction in BOP compared to non-smokers. This finding emphasises the negative effects of smoking on the bleeding response in the periodontium. Nevertheless, this finding is inconsistent with the results from the GLM analysis, which showed that there was only a visit and a patient effect on the whole-mouth clinical indices. This disagreement could be explained by the fact that the GLM analysis examined the interactions of several factors on the clinical parameters of all patients, while the current test is restricted to assess only the smoking effect on the whole-mouth clinical indices in a subgroup of patients. Taking these considerations into account, although this finding has a rational meaning it should be interpreted with caution. The site-specific analysis showed that post-treatment smokers had significantly less clinical improvement, in terms of PD, RAL, BOP and GCF volume reductions than did non-smokers. After treatment, smokers in the Q-SRP group retained lower serum antibody titres compared with non-smokers, and this was significant for *A. actinomycetemcomitans*. Significantly higher GCF antibody titres to *T. denticola* were found for smokers, indicating that these sites might still be challenged by this pathogen. No differences in the prevalence of putative periodontal pathogens were seen at R1 and R2 between smokers and non-smokers, both on a site- and a subject-basis.

At baseline, the effects of smoking on the clinical, immunological and microbiological parameters in the FM-SRP group were different from what was seen in the Q-SRP group. No significant baseline differences in the whole-mouth and site-specific clinical indices were found between smokers and non-smokers, as was the case with GCF volume. The pattern for lower serum antibody titres that was noted for smokers in the Q-SRP group was less clear in the FM-SRP group. The subject-based analysis showed no differences in the prevalence of specific microorganisms between smokers and non-smokers before and following FM-SRP therapy. However, significantly lower GCF antibody titres to *A. actinomycetemcomitans* and *P. intermedia* were seen for smokers

than non-smokers and of great interest was the finding that the prevalence of the homologous organisms was also significantly lower at sites of smokers. This strengthens the hypothesis that specific local antibody levels are related to antigenic load (Ebersole et al. 1982a). Between BAS and R2, significantly less reduction in GCF antibody titres to *P. gingivalis* was found for smokers compared to non-smokers, and this was the case for *T. denticola* between BAS and R1. Significantly lower percentage of sites positive for *A. actinomycetemcomitans* was seen in smokers than non-smokers in the FM-SRP group at R1. Following FM-SRP, selected sites of smokers showed significantly less PD and RAL reduction than those of non-smokers. No significant differences in the reduction of GCF volume were seen between sites of smokers and non-smokers in this treatment group.

These results reinforce the concept that smoking results in a compromised clinical outcome following treatment. Several studies confirm the present findings that smokers have less clinical improvement post-treatment than non-smokers (Preber et al. 1995; Kaldahl et al. 1996c; Haffajee et al. 1997a; Kinane and Radvar, 1997; Renvert et al. 1998). There is evidence that during maintenance smokers exhibit more periodontal destruction (Kaldahl et al. 1996b; Boström et al. 1998). The present data agree with other reports which found no differences in the presence of pathogens in supra- or subgingival plaque between smokers and non-smokers after treatment (Preber et al. 1995; Boström et al. 1998; Renvert et al. 1998). However, although the present study failed to show any pre- and post-therapy differences in the subgingival microflora between smokers and non-smokers when all subjects (in particular Q-SRP subjects) were analysed, differences in the site-prevalence of specific organisms were found between smokers and non-smokers in the FM-SRP group.

6.2.3 Comparison of smokers before and after Q-SRP and FM-SRP

Quirynen et al. (2000) examined three groups of patients. The two test groups received FM-SRP with the adjunctive use of chlorhexidine (Fdis) and without (FRp), whereas the control group received Q-SRP at two-weekly intervals. Each group comprised 12 patients and, although the authors reported that groups were well balanced for smoking habits, the Fdis group included 3/12 (25.0%) smokers, the FRp group 3/12 (25.0%) smokers, while the control group included 5/12 (41.5%) smokers. That study showed

that the smoking effect on the treatment outcome was unclear, although most of the smokers showed the least gain in attachment levels.

The findings of the current study demonstrated that smoking and treatment approach (Q-SRP and FM-SRP) over a period of six months had a significant effect on PD and RAL of the selected sites. Although this three-way interaction is complicated and difficult to interpret and may not be clinically significant, it appears to be that Q-SRP non-smokers had the greatest PD reduction between BAS and R1 compared to the others.

In order to clarify whether Q-SRP and FM-SRP had different effects on the clinical, immunological and microbiological parameters of smokers and non-smokers, these parameters were compared between smokers in the Q-SRP group and those in the FM-SRP group. This analysis found no significant differences in any clinical index at baseline between smokers in the Q-SRP and the FM-SRP groups. FM-SRP seemed to result in significantly lower bleeding scores at R2 and higher antibody titres to *P. intermedia* at R2. The reason why FM-SRP causes lower bleeding scores in smokers at six months compared to Q-SRP is difficult to explain and may be a spurious statistical finding.

CHAPTER 7

RELATIONSHIP BETWEEN SEVERITY OF PERIODONTAL DISEASE AND IMMUNOLOGICAL AND MICROBIOLOGICAL PARAMETERS

7. Relationship between severity of periodontal disease and immunological and microbiological parameters

7.1 Results

7.1.1 Correlation between immunological parameters and whole-mouth clinical indices

Serum IgG antibody titres (EU) to tested organisms were correlated negatively with baseline whole-mouth clinical indices, PD and No. of sites $\geq$ 5 mm and this was statistically significant for *A. actinomycetemcomitans* only (R=-0.49, p=0.001) (data not shown). In addition, IgG avidity (M at ID_{50}) tended to correlate negatively with baseline PD and No. of sites $\geq$ 5 mm, but this observation failed to reach statistical significance for any of the test bacteria.

In order to clarify these results and also compare the changes in clinical indices and changes in immunological and microbiological parameters, the subjects of this study were categorised as moderate and advanced diseased periodontitis patients and as having moderate and greater clinical improvement post-treatment. Then the immunogical and microbiological responses were compared in these patient subgroups and the results are discussed in the following section.

7.1.2 Relationship between severity of periodontal disease and immunological and microbiological parameters

Systemic immunological parameters (serum IgG titres and avidity) and microbiological parameters (percentage of patients positive for a species) were compared between subjects with advanced and subjects with moderate periodontal disease at baseline (Table 75). Serum IgG titres (EU) appeared to be higher in patients with shallower pockets (mean PD $\leq$ median) than those with deeper pockets (mean PD > median) and this finding was statistically significant for *A. actinomycetemcomitans* ($p<0.005$) and close to statistical significance for *P. intermedia* (p=0.055). Similarly, the patients with shallower pocket depths tended to have greater IgG avidity (M at ID_{50}) to the majority of the organisms tested than the latter patients, but this observation failed to reach statistical significance. *A. actinomycetemcomitans* was found to be more prevalent in patients with shallower than deeper pockets ($p<0.01$), but no significant differences for the other bacteria were found.

Table 75. Baseline immunological and microbiological parameters in moderately and advanced diseased periodontitis patients.

N_A=21 N_B=21		T. *P.g.* (EU)	p-value	T. *A.a.* (EU)	p-value	T. *P.i.* (EU)	p-value	T. *T.d.* (EU)	p-value	T. *B.f.* (EU)	p-value
PD(mm)	A	175 (59, 1566)	0.137	215 (52, 2544)	<0.003	333 (119, 973)	0.055	15 (9, 162)	0.182	124 (22, 1391)	0.102
	B	73 (20, 300)		20 (11, 66)		138 (35, 351)		12 (4, 37)		25 (11, 483)	
No. sites≥5mm	A	175 (64, 1566)	0.144	140 (52, 2544)	<0.008	333 (92, 973)	0.102	14 (9, 162)	0.279	124 (18, 1391)	0.247
	B	73 (25, 300)		20 (11, 73)		170 (26, 369)		14 (5, 37)		25 (13, 483)	
		Av. *P.g.* (ID_{50})	p-value	Av. *A.a.* (ID_{50})	p-value	Av. *P.i.* (ID_{50})	p-value	Av. *T.d.* (ID_{50})	p-value	Av. *B.f.* (ID_{50})	p-value
PD(mm)	A	0.48 (0.36, 0.63)	0.392	0.42 (0.36, 0.66)	0.314	0.93 (0.60, 1.05)	0.888	0.31 (0.27, 0.48)	0.385	0.45 (0.27, 0.66)	0.606
	B	0.45 (0.28, 0.62)		0.42 (0.30, 0.54)		0.83 (0.66, 1.00)		0.31 (0.22, 0.46)		0.41 (0.31, 0.58)	
No. sites≥5mm	A	0.47 (0.36, 0.60)	0.641	0.48 (0.35, 0.66)	0.296	0.91 (0.65, 1.05)	0.782	0.32 (0.29, 0.48)	0.242	0.45 (0.27, 0.63)	0.979
	B	0.45 (0.26, 0.67)		0.42 (0.30, 0.52)		0.83 (0.66, 1.00)		0.30 (0.22, 0.46)		0.42 (0.32, 0.59)	
		*P.g.**	p-value	*A.a.**	p-value	*P.i.**	p-value	*T.d.**	p-value	*B.f.**	p-value
PD(mm)	A	43.0	0.525	52.0	0.009	52.0	0.346	67.0	0.147	86.0	0.232
	B	33.0		14.0		67.0		86.0		100.0	
No. sites≥5mm	A	43.0	0.525	52.0	0.009	52.0	0.346	67.0	0.147	86.0	0.232
	B	33.0		14.0		67.0		86.0		100.0	

Median (Q1, Q3); T. = serum IgG titres; Av. = IgG avidity; *% of positive patients for a species

P-values compare moderately (A) and advanced (B) periodontitis patients

A : patients with mean (No. sites ≥ 5mm & PD) ≤ median; B : patients with mean (No. sites ≥ 5mm & PD) > median.

Between BAS and R2, the reduction in serum IgG titres seemed to be greater in patients with moderate clinical improvement (mean ΔPD ≤ median) than those with greater clinical improvement (mean ΔPD > median) and this finding was significant for *P. gingivalis* ($p<0.05$) and *B. forsythus* ($p<0.05$) and close to significance for *P. intermedia* ($p=0.054$) (Table 76). In the former patients IgG avidity to *T. denticola* decreased, whereas in the latter patients avidity increased and this difference between the two categories of patients was significant ($p<0.01$). Significantly greater reduction in the prevalence of *T. denticola* was noted for patients with the large than the moderate clinical improvement ($p=0.01$).

In a similar way, IgG titres appeared to be higher in patients with fewer pockets ≥ 5 mm ((mean No. of sites ≥ 5 mm) ≤ median) than those with more deeper pockets ((mean No. of sites ≥ 5 mm) > median) and this finding was statistically significant for *A. actinomycetemcomitans* ($p<0.01$) (Table 75). In addition, antibody avidity to all pathogens tended to be greater for the former patients but without reaching statistical significance. *A. actinomycetemcomitans* was found to be more prevalent in patients with fewer deep pockets ($p<0.01$). A greater reduction in serum IgG titres was found in patients who showed moderate clinical improvement ((mean ΔNo. of sites ≥ 5 mm) ≤ median) than those with large clinical improvement ((mean ΔNo. of sites ≥ 5 mm) > median) (Table 76). This was statistically significant for *P. gingivalis* ($p<0.05$) and *P. intermedia* ($p<0.05$) and close to significance for *T. denticola* ($p=0.054$). No significant differences in IgG avidity were found between the two categories of patients. A greater reduction in the prevalence of *T. denticola* was noted for the patients with the greater reduction in the number of sites ≥ 5 mm ($p<0.05$).

A comparison between the severity of disease and immunological and microbiological parameters was also made at a site-specific level, comparing the GCF IgG titres and the prevalence of the bacteria at sites of deep and moderately deep pocket depth. The subgingival microflora was compared between deep periodontal pockets (mean PD ≥ 7 mm) and moderately deep pockets (mean PD < 7mm) and no significant differences in the prevalence of the putative pathogens were found between the two categories of sites (Table 77). The comparison of BOP between deep and moderately deep pockets revealed higher BOP in the deep pockets than the moderately deep (84.4% versus 79.4%), but this finding was not statistically significant (Table 77). A higher percentage

Table 76. Comparison of changes in whole-mouth clinical indices and changes in immunological and microbiological parameters (BAS-R2).

N_A=20 N_B=19		ΔT. *P.g.* (EU)	p-value	ΔT. *A.a.* (EU)	p-value	ΔT. *P.i.* (EU)	p-value	ΔT. *T.d.* (EU)	p-value	ΔT. *B.f.* (EU)	p-value
PD(mm)	A	70 (15, 731)	0.043	33 (-3, 872)	0.086	50 (-10, 288)	0.054	6 (1, 67)	0.140	31 (1, 659)	0.033
	B	1 (-32, 143)		6 (-7, 34)		6 (-58, 54)		2 (-2, 9)		0 (-5, 12)	
No. sites≥5mm	A	96 (11, 1093)	0.020	33 (-6, 939)	0.094	50 (-14, 363)	0.040	10 (1, 116)	0.054	20 (-2, 518)	0.217
	B	1 (-33, 54)		6 (-6, 25)		6 (-55, 43)		2 (-5, 7)		0 (-4, 38)	
		ΔAv. *P.g.* (ID_{50})	p-value	ΔAv. *A.a.* (ID_{50})	p-value	ΔAv. *P.i.* (ID_{50})	p-value	ΔAv. *T.d.* (ID_{50})	p-value	ΔAv. *B.f.* (ID_{50})	p-value
PD(mm)	A	-0.02(-0.17, 0.16)	0.332	-0.09 (-0.34, 0.06)	0.361	0.06 (-0.20, 0.19)	0.431	0.02 (-0.04, 0.07)	0.006	0.0 (-0.16, 0.06)	0.527
	B	-0.06 (-0.26, 0.04)		-0.03 (-0.17, 0.02)		0.01 (-0.06, 0.07)		-0.08 (-0.17, 0.0)		0.0 (-0.11, 0.03)	
No. sites≥5mm	A	-0.02 (-0.21, 0.06)	0.898	-0.09 (-0.33, 0.03)	0.301	0.03 (-0.21, 0.12)	0.734	0.01 (-0.07, 0.07)	0.072	-0.03 (-0.12, 0.05)	0.943
	B	-0.06(-0.25, 0.11)		0.0 (-0.19, 0.04)		0.04 (-0.04, 0.12)		-0.04 (-0.16, 0.01)		0.0 (-0.16, 0.05)	
		Δ *P.g.**	p-value	Δ *A.a.**	p-value	Δ *P.i.**	p-value	Δ *T.d.**	p-value	Δ *B.f.**	p-value
PD(mm)	A	45.0	0.224	40.0	0.093	30.0	0.431	45.0	0.011	55.0	0.113
	B	26.0		16.0		42.0		84.0		79.0	
No. sites≥5mm	A	36.0	0.945	36.0	0.288	27.0	0.201	50.0	0.037	55.0	0.068
	B	35.0		18.0		47.0		82.0		82.0	

Median (Q1, Q3); ΔT. = changes in serum IgG titres; ΔAv. = changes in IgG avidity; *% of reduction in patients positive for a species

P-values compare patients with moderate (A) and large (B) clinical improvement

A : patients with mean (ΔNo. sites ≥ 5mm & ΔPD) ≤ median; B : patients with mean (ΔNo. sites ≥ 5mm & ΔPD) > median.

Table 77. Microbiological and clinical parameters in moderately deep and deep periodontal sites.

$N_A = 123$ $N_B = 36$		*P.g.**	p-value	*A.a.**	p-value	*P.i.**	p-value	*T.d.**	p-value	*B.f.**	p-value	BOP*	p-value	Sup*	p-value
PD(mm)	A	34.0	0.4	21.0	0.4	41.5	0.1	45.5	0.6	82.0	0.3	79.4	0.5	59.6	0.05
	B	28.0		28.0		28.0		41.6		75.0		84.4		78.0	

*% of positive sites

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

BOP: bleeding on probing; Sup; suppuration

P-values compare moderately deep (A) and deep (B) periodontal sites

A : sites with mean PD < 7 mm; B : sites with mean PD ≥ 7 mm.

Table 78. GCF IgG titres and GCF volume in moderately deep and deep periodontal sites.

$N_A = 123$ $N_B = 36$		*P.g.*	p-value	*A.a.*	p-value	*P.i.*	p-value	*T.d.*	p-value	*B.f.*	p-value	GCF volume*	p-value
PD(mm)	A	4.4 ± 0.8	0.430	1112.0 ± 820.0	0.030	2.0 ± 0.3	0.005	6.0 ± 1.8	0.420	1.2 ± 0.4	0.004	0.42 ± 0.3	0.35
	B	75.0 ± 45.0		9557.0 ± 9009.0		10.2 ± 6.0		7.7 ± 4.3		5.1 ± 2.1		0.46 ± 0.3	

Mean ± SEM; *mean ± sd

GCF IgG titres are expressed as EU/30sec, and GCF volume as µl/30sec

P.g.: P. gingivalis; *A.a.: A. .actinomycetemcomitans*; *P.i.: P. intermedia*; *T.d.: T. denticola*; *B.f.: B. forsythus*

BOP: bleeding on probing; Sup; suppuration

P-values compare moderately deep (A) and deep (B) periodontal sites

A : sites with mean PD < 7 mm; B : sites with mean PD ≥ 7 mm.

of deep pockets suppurated compared to moderately deep pockets (78.0% versus 59.6%), but this observation just failed to reach statistical significance (p=0.05) (Table 77).

Table 78 compares the GCF volume (µl / 30 sec) and GCF IgG titres (EU / 30 sec) to the five putative periodontal pathogens between deep pockets (mean PD $\geq$ 7mm) and moderately deep pockets (mean PD < 7mm). Deep pockets seem to give rise to more GCF, but this observation did not reach statistical significance. In addition, significantly higher GCF antibody titres to *A. actinomycetemcomitans* ($p<0.05$), *P. intermedia* ($p=0.005$) and *B. forsythus* ($p<0.005$) were found in deep pockets compared to moderately deep pockets. No significant differences in the GCF antibody titres to *P. gingivalis* and *T. denticola* were seen between sites of deep and moderately deep pocket depth.

7.2 Discussion

7.2.1 Relationship between severity of periodontal disease and immunological and microbiological parameters

At baseline there was a negative trend between serum antibody titres and clinical indices (PD and No. of sites $\geq$ 5mm). This observation was statistically significant for antibodies to *A. actinomycetemcomitans* only. In other words, the less the severity of disease, the higher the antibody levels were, implying that antibodies to the suspected pathogens play a protective role in the pathogenesis of periodontitis. However, the present data also showed that antibody levels were elevated in presence of a pathogen, implying that specific antibody levels reflect infection with a pathogen. Since *A. actinomycetemcomitans* was found to be more prevalent in subjects with shallower pockets and in subjects with more localised disease (fewer sites $\geq$ 5 mm), one would expect that serum antibody titres to this organism increase as pocket depth decreases. However, antibody avidity tended to follow the same pattern with antibody titres and correlated negatively with these clinical indices, suggesting that the shallower the pockets or the less widespread the disease, the higher the antibody avidity was. These findings strengthen the hypothesis that increased levels of antibodies that are of high avidity result in less disease severity in chronic periodontitis patients. The present data agree with previous reports which associated the failure of the host to elicit an immune

response to specific organisms with greater and more widespread periodontal disease (Ranney et al. 1982; Gunsolley et al. 1987).

It was of great interest to note that between baseline and R2 the smaller reductions in antibody titres were found for the subjects with the greater reduction in PD or number of deep sites. This observation implies two things. Firstly, it has been shown that PD reduction post-treatment is related directly to the initial PD with the greatest reduction seen for the deepest pockets (Knowles et al. 1979). The fact that antibody levels were higher in subjects with lower pocket depths and fewer deep sites may result in a greater reduction in antibody levels for these patients after treatment. The second and more likely possibility is that, despite the fact that antibody levels to all tested organisms decreased six months post-treatment, patients with a large clinical improvement retained higher antibody levels than those with a moderate clinical outcome. These patients showed a greater reduction in selected putative pathogens and this was significant for *T. denticola*. Antibody avidity to this pathogen was found to be significantly higher for patients with greater clinical improvement than those with less clinical improvement. These findings highlight the protective role of antibodies against bacterial infections and also support a role for *T. denticola* as a pathogen in periodontitis lesions (Simonson et al. 1988, 1990, 1992).

In conclusion, after treatment patients who had large clinical improvement also had higher antibody levels and higher antibody avidity against specific organisms compared to patients with moderate clinical improvement. This observation may reflect a maturation of the host response possibly accomplished by active immunisation during treatment. Alternatively, patients who can elicit a substantial immune response to suspected pathogens may be the ones with the lower pocket depths at baseline and the greater clinical improvement after treatment. There is evidence that seropositive patients respond better to treatment (Mouton et al. 1987; Mooney et al. 1995), and that the induction of an immune response in immunocompetent patients contributes to disease resolution after treatment (Ebersole et al. 1992). However, these inoculation benefits of root planing to the immune response were not evident when the immunological parameters were compared before and after therapy, regardless of the periodontal status of the patients. In general, a reduction in antibody titres to most of

the tested organisms was seen at six months, reflecting a reduction in the levels of pathogens.

The site-specific analysis showed that deep pockets had significantly higher GCF antibody titres to *A. actinomycetemcomitans*, *P. intermedia*, and *B. forsythus* than moderately deep pockets and tended to give rise to higher GCF volumes. No significant differences in the prevalence of suspected pathogens were found in deep versus moderate to deep pockets. Deep pockets have more pocket lining epithelium and it is assumed that they are more inflamed and have a greater reservoir of fluids and antibody producing plasma cells, giving rise to GCF and specific antibodies than shallow pockets. In accordance with this, Johnson et al. (1993) examined the GCF IgG titres to *P. gingivalis* in generalised aggressive periodontitis patients and found that GCF volume correlated with antibody titres, and that both of these parameters correlated with pocket depth. However, Mann (1963) suggested that inflammation had a stronger relationship to the amount of GCF than did the pocket depth. Conversely, Mooney and Kinane (1997) found lower GCF antibody titres in deep periodontal pockets than gingivitis sites, implying that increased consumption of local antibodies may take place in the deep pockets. Similar findings were found in an earlier study by the same investigators, but this report examined maintenance patients who received extensive periodontal therapy and therefore these results are not comparable with those in the current study (Kinane et al. 1993). However, there is evidence that there is no relationship between clinical disease status and elevated GCF antibody titres (Tew et al. 1985; Baranowska et al. 1989). The present data support the concept that PD is related to both GCF antibody levels and GCF volume, although significantly to the former only.

CHAPTER 8

GENERAL DISCUSSION

8. General discussion

8.1 Methodological considerations

8.1.1 Sample size

The present study followed the patients over a period of six months, and given time limitations and the fact that only one operator administered treatment, a size of forty patients was deemed suitable to give sufficient power for the analysis of microbiological, immunological and clinical data. Initially 60 patients were recruited, but ten subjects were excluded due to poor attendance and another eight subjects were excluded from the study due to antibiotic intake during the course of therapy. Therefore, the present data were based on 42 patients, with 20 and 22 of them comprising each treatment group (Q-SRP and FM-SRP, respectively).

This number of participants appears to agree with other studies and exceeds the number of patients in some of them (Lindhe et al. 1982a; Badersten et al. 1984a, 1984b; Isidor et al. 1984; Mouton et al. 1987; Lopatin et al. 1991; Kinane et al. 1993; Mooney and Kinane, 1997; Lie et al. 2001). The number of participants in each group exceeds that of a series of clinical trials, which assessed the efficacy of the one-stage full-mouth disinfection. The pilot studies of this laboratory were based on ten subjects, five in each treatment group (Quirynen et al. 1995; Vandekerckhove et al. 1996), and later studies from the same investigators examined a total number of 16 patients, eight in each group (Bollen et al. 1998), 40 patients, 20 in each group (Mongardini et al. 1999), and 36 subjects in the most recent study with 12 subjects in each treatment group (Quirynen et al. 2000).

8.1.2 Statistical considerations

There is conflicting data in the literature as to what the most appropriate unit of analysis should be. It has been suggested that the site should be used as the unit of analysis for identification of site-specific relationships (Haffajee et al. 1983a, 1983b; Haffajee and Socransky, 1986). It is argued that the evaluation of treatment outcome that assesses the whole-mouth clinical indices is inappropriate, since many sites are not diseased and these sites tend to negatively affect the statistical power of treatment effects. In contrast, other studies are in favour of using the subject as the unit of analysis and the sites as subunits, since sites within a subject may not act independently (Imrey, 1986; Fleiss et al. 1987; Sterne et al. 1988; Gunsolley et al. 1992).

The present study examined the efficacy of two treatment procedures and analysed clinical data collected from all sites in the mouth, and from four selected sites *per* patient, in order to assess systemic responses and site-specific responses to treatment. For the site-specific analysis an assumption was made that sites are statistically independent units without exhibiting intra-subject correlation. However, since both treatment procedures (Q-SRP and FM-SRP) had a systemic effect on the subjects, reliance on the GLM analysis of the full-mouth clinical indices and the GLM analysis of the pooled site-specific clinical indices *per* patient seems sensible. Efforts were made to minimise clinical measurement errors by recording valid and reproducible site-specific clinical indices. This resulted in similar findings after the analysis of the site-specific clinical indices when sites were used as independent experimental units, and after the analysis of the pooled site-specific clinical indices *per* subject when the subject was used as the experimental unit.

8.1.3 Site selection

Several studies estimated the necessary number of sites that need to be sampled from one patient to verify bacterial infection. It has been shown that two to four sites $\geq$ 5 mm need to be sampled to detect *P. gingivalis*, *P. intermedia*, and *B. forsythus*, while it has been claimed that more than 25 random sites are required to detect *A. actinomycetemcomitans* (Christersson et al. 1992; Gunsolley et al. 1992). These studies utilised indirect immunofluorescence and DNA probes for detecting the pathogens. The selection of the deepest pocket in each quadrant has been deemed sufficient both for the detection of *A. actinomycetemcomitans* as well as for the estimation of the highest cultivable count of the organism (Mombelli et al. 1994a). Another study showed that a species was not detected in 25% of the positive subjects if the four deepest pockets were sampled using a colony lift method and DNA probes (Haffajee and Socransky, 1992). The error in the false-negative detection of the species was higher for infrequently detected microorganisms, such as *A. actinomycetemcomitans* serotype b. This organism was not detected in 38% of positive subjects when the four deepest pockets were sampled. Nevertheless, this sampling strategy was found to be superior to other partial-mouth sampling methods in detecting the presence of a species (Mombelli et al. 1991; Haffajee and Socransky, 1992).

The present study assessed the presence or absence of selected pathogens in the four deepest sites *per* subject using the PCR method. Since PCR method is an extremely sensitive method for detecting specific organisms, the use of four deepest sites *per* subject, provided that their PD $\geq$ 5mm and they were on single-rooted teeth, seemed to be an efficient method to determine infection with a specific organism.

8.1.4 Plaque sampling for PCR analysis

In the present study plaque samples were collected using a sterile periodontal hand instrument, taking care to access the most apical part of the pocket. The plaque sample was collected with a single vertical stroke and it was a pooled sample including gingival, mid-pocket and apical samples. In a review by Tanner and Goodson (1986), different methodological approaches for plaque sampling were compared and some of these considerations are presented here. It has been shown that curettes can remove up to 90% of the subgingival plaque and this method of plaque collection is recommended if an estimate of the total pocket contents is required. Since hand instruments remove larger plaque samples than the other commonly used techniques (paper point, barbed – broach cannula, irrigation devices) they are more effective for sampling pockets with few organisms i.e., healthy sites or recently treated sites. Therefore, for studies in which disturbance of the ecosystem is not of major importance, this method appears to be the best sampling technique (Tanner and Goodson, 1986). Nevertheless, there is evidence that sampling *per se* has no statistically detectable effects on the composition of the microbial flora of subsequent samples from the same sites (Moore et al. 1982). Based on this finding, sampling using a curette may not significantly alter the subgingival ecosystem and therefore affect subsequent samples.

8.1.5 GCF sampling for ELISA

In the present study, GCF was collected with paper strips using Whatman filter paper cut in dimensions of 2 x 8 mm. The Whatman filter paper has been shown to be more advantageous than the Periopaper (Griffiths et al. 1988). That study demonstrated that 2 mm x 8 mm filter paper strips absorbed larger amounts of fluid while still remaining within the measuring scale of the Periotron 6000. The elution of serum-derived antibodies from Periotron filter papers was demonstrated to recover almost all of the antibody activity, compared with serum diluted directly into the antibody assay plates (Ebersole et al. 1984; Griffiths et al. 1988).

It has been suggested that calibration of the Periotron 6000 should be based on a range of volumes from very low to high, to be able to assess the quantities of fluid recovered from healthy or inflamed sites (Van der Bijl et al. 1986). In the present study, the calibration curve of the Periotron 6000 was split into two parts, the upper, including high volumes, and the lower, including lower volumes, and the two lines were fitted by regression (Lamster et al. 1985; Chapple et al. 1995). However, the range of volumes used to construct the lower part of the calibration curve in the present study was not as low as that used in the studies of Lamster et al. (1985) and Chapple et al. (1995). Nevertheless, the slope of linear regression found in this investigation was approximately 3.5 nl per unit for the lower part, and 6.3 nl per unit for the upper part of the curve, which is in agreement with other studies (Bickel and Cimasoni, 1984; Chapple et al. 1995).

It must be stressed that in the present study GCF antibody titres were expressed as ELISA units / 30 sec sample. These were independent of the volume of the GCF sample. As a result, the calculation of the GCF antibody levels were subjected to less variation with respect to the calibration of the Periotron 6000 and to evaporation of the fluid that may occur, regardless of the sampling technique used. The present results showed a large site-to-site variability in GCF antibody titres. In general, the levels of GCF antibodies were low for the majority of putative pathogens. One possible reason for the low values of GCF antibody titres is that GCF was eluted into 1 ml buffer for the ELISA analysis against five putative periodontal pathogens, which could result in a large dilution of the fluid's constituents. As noted in materials and methods, because GCF was eluted into 1 ml (1/1000 dilution) and the ELISA was standardised with a 1/200 dilution of the control serum, GCF titres should have been multiplied by five. However, even when this correction was made the GCF antibody titres were still low.

8.1.6 Periodontal probing

Listgarten et al. (1980) reviewed problems associated with probing and showed that a variety of factors seemed to influence the probing measurements, such as positioning of the probe, anatomic features of the tooth surface and the force exerted on probing. Histological evidence showed that errors related to periodontal probing appeared to not exceed 1.5 mm in the worst case (Listgarten, 1980). PD measurements are known to

depend on the probing force (Mombelli and Graf, 1986). Inflamed tissues offer less resistance to probe penetration than clinically healthy tissues (Armitage et al. 1977; Listgarten, 1980; Fowler et al. 1982), and a decrease in the variability of probing attachment levels was found after periodontal therapy (Badersten et al. 1984c).

Several studies have assessed the efficacy of constant force electronic probes in making reproducible recordings and have compared this technique with conventional probing. There is evidence that measurements obtained with an electronic probe were significantly superior to those obtained with a manual probe (Magnusson et al. 1988), while other studies failed to show any significant differences between manual and standardised probing (van der Velden and de Vries, 1980; Quirynen et al. 1993). A high correlation has been shown to exist between manual and electronic probing, but pocket depths recorded with a conventional probe were deeper than those recorded with an electronic probe (van der Velden and de Vries, 1980; Magnusson et al. 1988; Osborn et al. 1992; Quirynen et al. 1993). A further study showed that the percentage of agreement between the two techniques tended to decrease as pocket depth increased, although it was impossible to determine which technique recorded the periodontal status most accurately (Kalkwarf et al. 1985). In another study, both examiners were shown to be able to reproduce their measurements to within ± 1.0 mm on more than 96% of occasions with manual probing and on 100% of occasions with the constant-force probe (Walsh and Saxby, 1989). This was also the case in approximately 90% occasions when an electronic probe was used (Badersten et al. 1984c).

The main difference in reproducibility of the recordings taken with a manual probe compared with an electronic probe is the difficulty in applying a standard force when using a manual probe. In addition, the fact that measurements with a manual probe are recorded to the nearest mm while the constant force probe measurements are read to the nearest 0.1 or 0.2 mm also contributes to the disagreement between the two techniques. With this in mind, in the current study periodontal probing was performed using a manual probe for the whole-mouth PD and AL recordings and an electronic pressure sensitive probe (the Florida probe) for PD and RAL measurements at selected sites. The use of the Florida probe excluded errors, such as variations in probing force and inaccuracies with visual readings due to impaired vision, mainly pre-treatment. In the present study one examiner performed probing and therefore inter-operator variability

did not exist. It should be noted, however, that manual probing is a classical method of probing and has been widely used over the years and there are merits to this method. It is a simple procedure in the clinical setting and, additionally an experienced examiner can use tactile sensation to overcome problems that may obscure accurate probing, such as anatomic features of the root surface or the presence of subgingival restorations and calculus.

8.2 Conclusions

8.2.1 Comparison of Q-SRP and FM-SRP treatments

The GLM analysis showed no significant effects of smoking and / or treatment strategy on the whole-mouth clinical indices (PD, AL, BOP and No. of sites≥5 mm). However, when the selected sites were considered a significant combined effect of smoking and treatment strategy was seen on PD and RAL over the six month period. When the two treatment groups were compared at a site-specific level, the Q-SRP group was found to have a significantly greater PD reduction at R1 in moderately deep pockets and a significantly smaller gain in RAL at R2 in deep pockets compared to the FM-SRP group. No differences in any other clinical index were found between the two treatment strategies.

There were no significant differences in IgG antibody avidity between the two treatment groups before and after therapy. However, FM-SRP resulted in a significantly greater reduction in serum antibody titres to *P. intermedia* and *T. denticola* between baseline and R1 and higher GCF antibody titres to *T. denticola* at R1 compared to Q-SRP. In addition, significantly lower site prevalence of *P. intermedia* was seen at R1 for the FM-SRP group and a greater reduction was noted in subject prevalence of *T. denticola* at R1 and R2 from baseline for the FM-SRP group compared to the Q-SRP group. These findings imply that changes in immunological parameters parallel changes in microbiological parameters. However, the clinical interpretation of these results is difficult.

The data from the present study showed no evidence of microbial re-colonisation or clinical deterioration in the first scaled quadrant (quadrant one) compared to quadrant four that was scaled last. The former quadrant was treated six weeks before quadrant four was root planed when Q-SRP treatment was completed. This finding tends to

contradict the hypothesis of Quirynen et al. (1995) that bacterial contamination may occur during the active phase of Q-SRP treatment, re-infecting already treated sites from the remaining untreated pockets.

During consecutive sessions of Q-SRP at two weekly-intervals, pockets in the untreated quadrants showed significant improvements in clinical indices (PD, MGI, PI, Sup). This may have been due to improved oral hygiene measures or a host-induced effect by Q-SRP. Nevertheless, these improvements were smaller compared to changes seen after the completion of treatment. The assessment of the humoral immune response indicated that reductions in antibody titres and increases in antibody avidity occurred during the active phase of treatment with reference to baseline, but this was significant for some of the tested organisms. These findings indicate that reductions in the antigenic load occurred at the early stages of treatment and these microbial changes appeared to have a significant effect on the host response. No significant differences in serum antibody levels or antibody avidity were found between the two treatment groups during therapy to support the hypothesis of a strong immunological reaction induced by bacteraemia after the one-day full-mouth treatment as suggested by Quirynen et al. (2000).

Despite the fact that FM-SRP resulted in higher pain scores and higher frequency of labial herpes, this treatment approach seemed to be well tolerated by patients. FM-SRP patients considered this approach to be more practical, since fewer treatment visits are required to complete this therapy than the classical treatment of consecutive sessions of root planing. However, it should be emphasised that in the current study FM-SRP patients were seen as frequently as Q-SRP patients to receive equal amounts of motivation and oral hygiene instructions.

8.2.2 Effect of smoking

The site-based analysis revealed that smokers had significantly lower PD, MGI and GCF volume, and a tendency for higher RAL and lower BOP at baseline compared to non-smokers. Significantly lower GCF antibody titres and a tendency for lower serum antibody titres were found in smokers than non-smokers at baseline. No significant differences in the subgingival microflora were noted between smokers and non-smokers at baseline. These results imply that smoking depresses the inflammatory response of

the periodontium to plaque challenge and has a tendency to modify the immunological profile of periodontitis patients.

A significantly smaller PD reduction and significantly less RAL gain were noted in smokers compared to non-smokers after both treatment strategies, which emphasises the negative effects of smoking on the treatment outcome. The GLM analysis showed that smoking and treatment strategy (Q-SRP and FM-SRP) had a significant effect on the clinical indices (PD and RAL) of the selected sites over the course of treatment. It was interesting to note that smokers and non-smokers from both treatment groups showed a different pattern in the clinical, immunological and microbiological responses before and after treatment. In order to clarify this observation an attempt was made to compare smokers in the Q-SRP and FM-SRP groups. No significant differences in any clinical index were found between Q-SRP smokers and FM-SRP smokers at baseline, while a greater BOP reduction was seen after treatment (at R1 and R2) for smokers in the FM-SRP group compared to the Q-SRP group. This finding is difficult to interpret, based on their immunological and microbiological differences before and after treatment and the small numbers in the study.

8.2.3 Immunological and microbiological responses

The current study showed significant reductions in serum antibody titres with a concomitant decrease in the prevalence of putative periodontal pathogens. This finding implied that post-SRP antibody titres decreased as the microbial burden lessened and these changes were more pronounced at six months. Although, antibody avidity tended to increase post-scaling, this observation failed to reach statistical significance for most of the bacteria tested. In conclusion, treatment resulted in lower antibody levels, but of similar avidity for the majority of the microorganisms.

The finding that antibodies reflect the presence of the homologous organisms subgingivally is confirmed by the fact that subjects who harboured a specific microorganism had elevated serum antibody titres to that species, and this was statistically significant for *P. gingivalis* and *A. actinomycetemcomitans*. In addition, GCF antibody levels were significantly higher at sites positive for *A. actinomycetemcomitans*. The subjects that became negative for a pathogen post-treatment tended to have a greater reduction in serum antibody levels. This finding was

significant for *A. actinomycetemcomitans* and those subjects who no longer harboured *A. actinomycetemcomitans* after treatment, also showed a greater clinical improvement compared to the subjects who had no change or became positive for this species.

8.2.4 Relationship between severity of disease and immunological and microbiological parameters

At baseline, subjects with more advanced periodontal disease demonstrated lower serum antibody titres than those with moderate periodontitis. After treatment, subjects with large clinical improvement exhibited a smaller reduction in serum antibody levels compared to subjects with moderate clinical improvement. For *T. denticola* this observation just failed to reach statistical significance ($p=0.054$), but coincided with a significantly greater increase in antibody avidity and a significantly greater reduction in the prevalence of this pathogen post-treatment. These findings suggest that subjects who are able to mount a specific immune response to suspected pathogens exhibit less severe disease and also show a better clinical outcome and greater reductions in the prevalence of these species. This strengthens the hypothesis that antibodies are protective against disease and also that *T. denticola* plays an important role in the pathogenesis of periodontitis.

A. actinomycetemcomitans was found to be more prevalent is subjects with less severe periodontal disease in contrast to *T. denticola* and *B. forsythus*. This finding is consistent with *A. actinomycetemcomitans* being a facultative anaerobic organism, unlike the latter species which are fastidious anaerobes.

At a site-specific level, deep sites exhibited higher GCF antibody titres (significantly for *A. actinomycetemcomitans*, *P. intermedia* and *B. forsythus*) and also a tendency for higher GCF volume compared to moderately deep sites. This observation suggests that deeper sites are more inflamed and give rise to higher levels of specific antibodies in the GCF than shallower pockets. In addition, a higher percentage of deep pockets was found to suppurate and also sites that harboured *A. actinomycetemcomitans* and *B. forsythus* had a higher frequency of suppuration.

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List of publications

Abstract presentations:

Apatzidou D.,* Kinane D.F., Riggio M.P (2001) Effect of periodontal therapy on the antibody response and subgingival microflora. *Journal of Dental Research* **80 (Spec. Issue)**, 17

Apatzidou D.A.,* Kinane D.F. (2001) Clinical findings following quadrant root planing versus full mouth root planing. *Journal of Dental Research* **80 (Spec. Issue)**, 185.

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Apatzidou D.A.,* Riggio M.P., Kinane D.F. (2002) Clinical, immunological and microbiological responses to periodontal therapy in smokers and non-smokers with periodontal disease. *Journal of Dental Research* (in press).

The following abstract is not directly related to the current study:

Marshall G.J., Kinane D.F., Lowe G.D.O., Lösche W., Rumley A., Apatzidou D. (2001) Changes in rheological cardiovascular risk factors after periodontal therapy. *Annals of Periodontology* (in press).

Effect of periodontal therapy on the antibody response and subgingival microflora. D. APATZIDOU,* D.F. KINANE, M.P. RIGGIO (Glasgow Dental Hospital & School, Glasgow, UK).

Previous studies have shown that periodontal treatment stimulates the host immune response to produce antibodies of higher avidity and lower antibody titres. The aim of this study was to examine the clinical, microbiological and immunological effects of four sessions of quadrant root planing at two-week intervals. 20 patients with advanced periodontitis were followed over a period of 6 months. Clinical measurements and subgingival plaque samples were taken from 4 selected sites at baseline, reassessment one (RAS1) and reassessment two (RAS2). Moreover, full mouth pocket charts and serum samples were collected at these visits. Antibody titre was assayed by ELISA and avidity was measured by thiocyanate dissociation against five putative periodontal pathogens, *P. gingivalis*, *A. actinomycetemcomitans*, *P. intermedia*, *T. denticola* and *B. forsythus*. The presence of the homologous bacteria in subgingival plaque samples was determined by PCR. Periodontal treatment resulted in significant improvements for all clinical parameters with the more marked differences detected at RAS2, ($p<0.05$). Immunoglobulin G (IgG) titres decreased after treatment but did not reach significance for any of the five tested bacteria, whereas IgG avidities increased post-treatment with a significant increase in *P. gingivalis* ($p<0.05$). Microbiological data indicated reductions of all pathogens detected in subgingival plaque samples after treatment. These results indicate that scaling and root planing affects the dynamics of the immune response and reduces the subgingival pathogenic microflora.

Clinical findings following quadrant root planing versus full mouth root planing. D.A. APATZIDOU,* D.F. KINANE (Glasgow Dental Hospital & School, Glasgow, UK).

Full mouth root planing (FMRP) has recently been claimed to be superior to quadrant root planing (QRP) in terms of clinical and microbiological responses. The aim of this study was to examine the clinical effects of full mouth root planing within the same day, compared to the more typical therapy of four sessions of quadrant root planing at two weekly intervals. 40 patients with advanced periodontitis were randomly allocated to QRP (n=20) and FMRP (n=20) groups and were followed intensively over a period of 6 months. Clinical measurements were taken from 4 selected sites at baseline and at the reassessment visits at 3 months (R1) and 6 months (R2). Full mouth pocket charts and serum samples were also collected at these visits. Both treatment modalities resulted in statistically significant improvements for all clinical parameters with the more marked differences detected at R2. At baseline, the mean and standard deviation for the pocket depth (PD) was 4.3 ±0.6mm for FMRP, and 4.5 ±0.7mm for QRP. The mean PD reduction was significant for both groups ($p<0.005$) at R1 (FMRP: 1.5 ±0.5mm; QRP: 1.7 ±0.7mm) and at R2 (FMRP: 1.7 ±0.6mm; QRP: 1.9 ±0.7mm). All pockets equal or greater than 5mm were significantly reduced at R1 (FMRP: 70%; QRP: 72%) and at R2 (FMRP: 84%; QRP: 86%). No differences in any of the clinical indices were seen between the two groups after treatment. In conclusion, both treatment strategies appear to be equally effective in the treatment of advanced periodontitis.

Antibody dynamics following quadrant root planing versus full mouth root planing. D.A. APATZIDOU, D.F. KINANE* (Glasgow Dental Hospital & School, Glasgow, UK).

Full mouth root planing (FMRP) has recently been claimed to be superior to quadrant root planing (QRP) in terms of clinical and microbiological responses. The aim of this study was to examine the immunological effects of full mouth root planing within the same day compared to the more typical therapy of four sessions of quadrant root planing at two weekly intervals. 40 patients with advanced periodontitis were followed over a period of 6 months. Clinical data were recorded before and after treatment and serum samples were collected at these visits and at two week intervals during the active phase of therapy for both QRP (n=20) and FMRP (n=20) groups. Antibody titre was assayed by enzyme-linked immunosorbent assay (ELISA) and avidity was measured by thiocyanate dissociation against five putative periodontal pathogens: *P. gingivalis*; *A. actinomycetemcomitans*; *P. intermedia*; *T. denticola* and *B. forsythus*. Overall, Immunoglobulin G (IgG) titres decreased after treatment, with a statistically significant decrease in antibody titre to *T. denticola* ($p<0.05$). In contrast, IgG avidities increased post treatment for all bacteria, with a statistically significant increase noted for *A. actinomycetemcomitans* ($p=0.05$). No significant differences in antibody titres or avidities were detected between the two treatment groups. In conclusion, both treatment strategies appear to have a similar impact on the magnitude and quality of the immune response.

Clinical, immunological and microbiological responses to periodontal therapy in smokers and non-smokers with periodontal disease.

Apatzidou D.A.,* Riggio M.P., Kinane D.F. University of Glasgow, Scotland, UK

Objectives: The aim of this study was to determine the clinical, microbiological and immunological profiles of smokers and non-smokers periodontitis patients before and after conventional non-surgical periodontal therapy.

Materials and Methods: 20 patients with moderate to severe periodontitis received mechanical debridement and were followed over 6 months. Clinical data were recorded and serum, gingival crevicular fluid (GCF) and subgingival plaque samples were collected before and after treatment. Antibody titres were assayed by enzyme-linked immunosorbent assay (ELISA) and avidity was measured by thiocyanate dissociation against five putative periodontal pathogens: *P. gingivalis*; *A. actinomycetemcomitans*; *P. intermedia*; *T. denticola* and *B. forsythus*. The presence of these bacteria in plaque was determined by polymerase chain reaction (PCR).

Results: Smokers showed significantly less gingival inflammation and GCF volume at baseline than non-smokers ($p<0.05$). Less pocket depth reduction, less attachment gain and a smaller reduction in GCF volume was seen in smokers compared with non-smokers after treatment ($p<0.05$). At baseline, serum IgG titres were lower for smokers ($p\leq0.05$ for *A. actinomycetemcomitans* and *P. intermedia*) while their change with treatment was similar for both subgroups. No significant differences in IgG avidity and subgingival microflora were seen between smokers and non-smokers before and after treatment.

Conclusion: Smoking has an adverse effect on the clinical outcome of periodontal therapy and appears to affect the immunological profile of periodontitis patients. Smoking reduces gingival inflammation and thus masks early signs and symptoms of periodontal disease.

Changes in rheological cardiovascular risk factors after periodontal therapy.

Marshall G.J., Kinane D.F., Lowe G.D.O., Lösche W., Rumley A., Apatzidou D.

This short-term interventional study aimed to detect changes in rheological risk factors for atheromatous disease following periodontal therapy. 40 patients with periodontitis (probing depths ≥5.5mm in at least 2 quadrants) were recruited. Resting blood pressure and a baseline venous blood sample were taken and clinical indices recorded. All patients received quadrant root planing and oral hygiene instruction and were reviewed 6-8 weeks after treatment was completed. Preliminary analysis of the first 33 completed patients confirmed that periodontal treatment gave a significant reduction in pocket depth ($P<0.0001$) and bleeding on probing ($P<0.0001$). There were significant rises in clotting factors VII and VIII after treatment ($P<0.05$). Factor IX also rose after treatment, but this change did not reach statistical significance ($P<0.066$). No other statistically significant changes in the rheological variables tested were noted, however there was a trend towards elevated levels of tissue plasminogen activator (TPA) and fibrin D-dimers after treatment. In contrast, serum from 33 patients were analysed for activity of the enzyme Platelet Activating Factor - Acetylhydrolase (PAF-AH) and revealed a decrease in enzyme activity after treatment ($P<0.001$). In conclusion, this preliminary analysis suggests that within 6–8 weeks of periodontal therapy there is a reduction in systemic PAF-AH activity and a perturbation of clotting factors VII, VIII and IX.

A further report on the effects of periodontal therapy after 6-9 months will follow and will inform us as to whether the effects seen post initial treatment are transient as for exercise and blood clotting factors, and drop during the stable maintenance period.