EVALUATION OF ADJUNCTIVE AND ALTERNATIVE TECHNIQUES IN PERIODONTAL THERAPY

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ABREVIATIONS

A.a.:	*Actinobacillus actinomycetemcomitans*
Adj SS:	adjusted sum of square
Adj MS:	adjusted mean square
AL:	attachment level
ANOVA:	analysis of variance
BOP:	bleeding on probing
CEJ:	cemento-enamel junction
CFU:	colony forming unit
CI:	95% confidence interval
d.f.:	degrees of freedom
EVA:	ethylene vinyl acetate
FAB:	Fastidious Anaerobe Broth
GCF:	gingival crevicular fluid
GI:	gingival index
GLM:	General Linear Model
kV:	kilovolt
LJP:	localised juvenile periodontitis
LPS:	lipopolysacharide
MGI:	Modified Gingival Index
MIC_{90}:	90% minimum inhibitory concentration
mJ:	millijoules
M.S.:	mean square
N:	Newton
Nd:YAG:	neodymium: yttrium, aluminium, garnet
nm:	nanometer
N.S.:	not significant
OHI-S:	simplified oral hygiene index
PD:	pocket depth
PD0:	baseline pocket depth
P.g.:	*Porphyromonas gingivalis*
P.i.:	*Prevotella intermedia*
PI:	Plaque Index
PMA:	papillary, marginal & attached gingiva index
pps:	pulses per second
S:	scaling and root planing alone
SD:	standard deviation
SEM:	scanning electron microscopy
SEM:	standard error of the mean
Seq SS:	sequential sum of square
S&Me:	scaling and root planing plus metronidazole gel
S&Mi:	scaling and root planing plus minocycline gel
S&T:	scaling and root planing plus tetracycline fibre
S.S.:	sum of squares
W:	watt

ΔPD: change in pocket depth
ΔAL: change in attachment level
δ_o: mean difference
σ: standard deviation

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DECLARATION

This thesis is the original work of the author.

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SUMMARY

Mechanical debridement of supragingival but predominantly subgingival bacterial plaque and calculus has been the mainstay of traditional treatment for periodontal disease. Practical difficulties and occasionally lack of response or recurrence of disease in some patients have made investigators search for more effective alternative or adjunctive methods of therapy. In this thesis, two modern approaches, local antimicrobial delivery systems, as adjuncts to scaling and root planing, and the Nd:YAG laser, as an alternative to scaling and root planing, were investigated.

The study designed to investigate subgingival antimicrobial systems was of randomised parallel design and sought to evaluate the efficacy of 3 locally delivered antimicrobial systems as adjuncts to scaling and root planing in the treatment of sites with persistent periodontal lesions. Fifty-four patients with 4 pockets ≥ 5 mm and bleeding on probing (BOP) and/or suppuration were randomised in 4 treatment groups including: scaling and root planing either alone (S) or plus application of 25% tetracycline fibre (S&T) or 2% minocycline gel (S&Mi) or 25% metronidazole gel (S&Me). All treatments resulted in significant improvement in pocket depth (PD), attachment level (AL), BOP and the Modified Gingival Index (MGI)

scores which were maintained until the end of the 6-month follow-up period, although some rebound towards baseline occurred in all groups at the 6-month visit. The improvements in clinical parameters were greater in the S&T group than other groups at any time point. At the 6-week visit, the pocket depth reduction (ΔPD) was significantly greater in the S&T group than in the S group (p=0.002). There was no significant difference between groups in ΔPD at 3 and 6-month visits. The difference between groups in improvement of AL (ΔAL) or BOP was not significant at any time point. The frequency of sites with suppuration reached zero only in the S&T group at the 6-week and 3-month visits. No serious adverse effects were observed or reported in any of the treatment groups. The S&T was the most time-consuming treatment. While all 3 locally applied antimicrobial systems appear to offer some benefit over scaling and root planing alone, S&T treatment demonstrated the greatest advantage in the treatment of persistent periodontal lesions, particularly at suppurating sites.

A further aim of the study was to evaluate the effect of smoking on the outcome of periodontal therapy. Regardless of the type of treatment, ΔPD and ΔAL were consistently greater in non-smoker subjects than smokers. The General Linear Model analysis was used for ΔPD and ΔAL to take into account, variations in the treatments, number of smokers per group and baseline pocket depth. There was

consistently a significant interaction between the 'smoking' and the 'baseline PD'. Further analysis using linear regression indicated that while there was a significant relationship between the baseline PD and the ΔPD or ΔAL among the non-smokers, a weak and insignificant relationship existed among the smoker subjects. These results strongly suggest that smoking is a factor to consider in the determination of prognosis of periodontal treatment, particularly in deep pockets.

The aim of the first laser study was to evaluate the effects of Nd:YAG laser treatment on sub-gingival calculus, cementum and dentine, *in vitro* at different power settings and durations. Eight different laser treatment settings were tested on subgingival calculus, cementum and dentine specimens and were assessed using scanning electron microscopy (SEM). Micrographs were taken from each treated site at x100 and x750 magnifications. An arbitrary scale (from 0 to 3) was used to score the degree of damage caused by the laser. Generally, the laser caused greater damage on calculus than either cementum or dentine specimens, although in cases of complete ablation of calculus, the underlying cementum was ablated too. This may limit selective calculus removal without damaging the underlying dental tissues. Three-way analysis of variance showed that for calculus, the power setting, pulse repetition, and the duration of exposure, contributed independently to the

mean damage score in an additive way. The results also showed that there was variability in susceptibility of different teeth and different parts of each tooth, which was true for calculus, cementum and dentine. This variability may preclude the safe and predictable removal of calculus by specific laser settings.

The aim of the second laser study was to determine whether the Nd:YAG laser energies of 50 and 80mJ at 10pps were capable of improving the clinical parameters associated with periodontal disease. These energy settings were chosen as previous reports indicated that higher values would damage root surfaces and that 80mJ had an *in vitro* bactericidal effect. Eighty periodontally affected sites in teeth scheduled for extraction from 11 patients with adult periodontitis were randomly placed in one of the following 4 treatment groups: 1) laser treatment at 50mJ, 10pps for 3 minutes; 2) laser treatment at 80mJ, 10pps for 3 minutes; 3) scaling and 4) untreated control. After 6 weeks, only the scaling group showed a significant reduction in pocket depth and BOP ($p<0.001$). The microbial samples taken immediately after scaling and 80mJ laser treatments showed a significant reduction in total anaerobic colony forming units compared to baseline ($p<0.01$), which was sustained only in the scaling group until week 6. SEM did not reveal any heat damage on the root surfaces. This study demonstrated that application of Nd:YAG laser pulses of 50mJ and 80mJ failed to improve

the clinical and microbiological parameters of periodontal disease.

While subgingivally delivered antimicrobial systems, may be useful adjuncts to root planing in selected sites with persistent pocketing, the Nd:YAG laser, despite the manufacturer's claims, was not found to be a useful alternative to root planing. At the lower energy settings no clinical benefit was noted, and at the higher energy settings the results suggested that the laser is potentially damaging to the root cementum and dentine.

Further research should be directed towards the development of resorbable antimicrobial delivery systems with improved substantivity and more convenient and less time-consuming insertion methods. Such a system, may reduce the duration and number of required visits, and thus will improve the patient compliance and acceptance. Laser research should be directed towards the development and clinical testing of laser wavelengths with selective absorption for bacteria and calculus and minimal absorption in the dental tissues.

CHAPTER I

INTRODUCTION

1.1 General introduction

Periodontal disease is among the most widely distributed diseases of mankind. Much effort has been expended to increase the knowledge of the pathogenesis of the disease. In spite of the rapidly expanding knowledge of the aetiological factors and pathways which interact with disease progression, a full understanding of the disease process has not yet been achieved. With an increase in the average life span, and a decrease in the prevalence of tooth loss due to caries, more teeth are now at risk of developing periodontal disease, and the need for skilful and knowledgeable prevention and therapy is increasing. Along with the development of new diagnostic tools, the ability of clinicians to diagnose and plan preventive and treatment strategies is improving. As the understanding of the pathogenesis of periodontal disease has evolved, these strategies have been developed and modified. While some of these strategies are no longer universally accepted, some others are still in the process of early development.

This thesis attempts to evaluate some of the recent therapeutic techniques developed for the treatment of periodontal disease. These new therapies may be adjuncts or alternatives to the established practice of root planing. These include local drug delivery systems and laser therapy, both of which have been suggested to act mainly by an antimicrobial action. The following literature review initially deals with definitions of

basic aspects of periodontology. Then basic concepts of periodontal treatment will be considered. Special consideration will be given to background research on local antimicrobial therapy and laser therapy. Finally, the clinical methods for assessing the outcome of periodontal treatment will be critically reviewed.

1.2 Definitions

1.2.1 The 'normal' periodontium: Clinical and histological appearance

Supporting structures of the teeth, known as the periodontium, are comprised of the alveolar bone, the periodontal ligament, the root cementum and the gingivae which together form a functional unit (Lindhe, 1989). The normal periodontium is clinically pale pink in colour, has a scalloped outline and a firm texture. It does not bleed on gentle probing, and fills the entire space between adjacent teeth (Wennström, 1988). In health, the depth of gingival sulcus is minimal. Histologically, no inflammatory infiltrate is present and only a few polymorphonuclear leucocytes can be seen within the connective tissue and junctional epithelium. In a normal periodontium the alveolar bone is located 1mm apical to the cementoenamel junction, but a clinically healthy periodontium may show tissue recession and reduced height of the alveolar bone. Furthermore, the classical microscopic picture of the 'normal' periodontium has recently been an issue of debate. Investigators have found

that an inflammatory infiltrate, comprising of both polymorphonuclear leucocytes and small round mononuclear cells is always present in gingival biopsies from clinically healthy gingivae (Seymour, Powell and Aitken, 1983; Brecx *et al.*, 1987). Therefore, the term 'clinically healthy' compared to 'normal' periodontium is preferable (Wennström, 1988).

1.2.2 Diseased periodontium: Clinical features and epidemiology

Gingivitis refers to pathological inflammatory changes which are limited to the gingivae, and are clinically manifested by a change in colour (redness), texture and appearance (swelling) of the gingivae, together with an increased tendency to bleeding on gentle probing. Periodontitis affects the deeper structures of the periodontium (bone, periodontal ligament and cementum), resulting in loss of periodontal support. It is frequently associated with the presence of periodontal pockets and bleeding on probing. Bone loss is the pathognomonic feature.

The world workshop in clinical periodontology (1989) classified different forms of periodontal disease as follows: 1) Adult periodontitis; 2) Early-onset periodontitis including: a) Prepubertal periodontitis (localized and generalized), b) Juvenile periodontitis (localized and generalized), and c) Rapidly progressive

periodontitis; 3) Periodontitis associated with systemic disease; 4) Necrotizing ulcerative periodontitis; and 5) Refractory periodontitis. However, there is considerable overlapping within the classification defined above. For example, the presence of a systemic disease such as diabetes may contribute to the development of a non-responsive so-called refractory periodontitis. HIV-associated periodontitis could be classified as periodontitis associated with systemic disease, although it is usually manifested by features of necrotizing ulcerative periodontitis. Furthermore, separation of the generalized form of juvenile periodontitis from rapidly progressive periodontitis adds confusion. Finally, the age of onset is sometimes difficult to determine retrospectively because quite often the patients are not aware of the presence of the disease until informed by their dentist. Periodontitis is a multifactorial, complex and chronic disease. Clearly, a better understanding of histopathological and epidemiological characteristics of the disease is needed before more clear-cut and well-defined classifications become available.

Early reports on the prevalence of periodontitis indicated that destructive periodontal disease affected a majority of the adult population above the age of 35-40 (Belting *et al.*, 1953; Sandler and Stahl, 1954; Marshal-Day *et al.*, 1955; Bossert and Marks, 1956; Russel, 1957; Schei *et al.*, 1959). Furthermore, from the above studies it was

concluded that the inevitable fate of an untreated gingivitis was the development of periodontitis (Schrep, 1964). During the 1980's a new epidemiological concept emerged, as a result of new surveys, using more well-defined criteria in screening the patients. The new findings were not consistent with the old concepts. Generally, it was found that a small percentage of the population over the age of 40 were affected by periodontal disease (Baelum, Fejereskov and Manji, 1988; Jenkins and Kinane, 1989; Youneyama *et al.*, 1988; Brown, Oliver and Löe, 1990; Hugoson, Laurell and Lundgren, 1992). Moreover, longitudinal studies on periodontitis populations revealed that a very small fraction of sites undergo attachment loss if left untreated (Lindhe, Haffajee and Socransky, 1983; Haffajee, Socransky and Goodson, 1983a; Jenkins, MacFarlane and Gilmour, 1988; Lindhe *et al.*, 1989). Papapanou *et al.* (1988) reported that over a 10 year period, only 7% of subjects over 25 years of age showed >3 mm attachment loss. Löe *et al.* (1986) conducted a 15 year study on a population who never practised oral hygiene measures. It was found that rapid progression of periodontal disease occurred in 8% of the total population. Collectively, the recent studies indicate that a subfraction not exceeding 10-20% of the whole populations show evidence of extensive periodontal disease.

Realisation of the fact that certain individuals, and even certain sites in general, are more susceptible to periodontal disease than others, has made investigators focus on risk factors and markers of this disease. Over the years many risk factors have been proposed. These include host factors and environmental factors. Host factors could either be local (e.g. anatomic and plaque retentive factors) or systemic factors. A variety of immunologic and endocrine disorders are associated with impaired host response and thus, may be associated with increased susceptibility to plaque - induced periodontal break-down (reviewed by Kinane and Davies, 1990). Although it has been documented that with increasing age, the prevalence of periodontal disease increases (Marshall-Day *et al.*, 1955), the age *per se* may represent a past history of exposure to the disease rather than an actual current susceptibility. Moreover, the increased progression of periodontal disease in the elderly may be due to the association of ageing with cumulative attachment loss (Haffajee *et al.*, 1991) or recession (which results in exposed roots with greater tendency for plaque retention) (Kinane and Davies, 1990). Stress may affect the host response by increasing the level of corticosteroids. Furthermore, it may also affect the patients oral hygiene habits and therefore may be a risk factor. Among the environmental factors the presence of some micro-organisms have been reported to have strong associations with periodontal disease (reviewed by

Haffajee and Socransky, 1994). Finally, smoking has been shown to affect various aspects of host immune response. Smoking may have an adverse effect on fibroblast function (Raulin *et al.*, 1988), chemotaxis and phagocytosis by neutrophils (Kenny *et al.*, 1977; Kraal *et al.*, 1977), immunoglobulin production (Holt, 1987; Johnson *et al.*, 1990) and induction of peripheral vasoconstriction (Clarke, Shephard and Hirsch, 1981). Epidemiologic evidence indicates that cigarette smoking is a stronger risk indicator for the presence of periodontal disease than any of the 5 most suspected periodontal pathogens i.e. *Actinobacillus actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Prevotella intermedia (P.i.), Eikenella corrodens* and *Fusobacterium nucleatum* (Stoltenberg *et al.*, 1993).

1.2.3 Diseased periodontium: Aetiology and pathogenesis

Periodontal disease is a disease of the supporting tissues of the teeth. Evidence indicates that periodontal disease is the result of interactions between bacterial plaque and the host response. If the pathological alteration is limited to the gingiva, it is called gingivitis, and if it leads to destruction of the supporting connective tissue and apical migration of the junctional epithelium, it is referred to as periodontitis.

1.2.3.1 Gingivitis

Gingivitis refers to inflammatory lesions that are confined to the gingiva. The main aetiological factor in gingivitis is dental plaque (Löe, Theilade and Jensen, 1965). In fact, other factors have only a secondary role by either enhancing plaque accumulation or increasing host susceptibility to the plaque. Page & Schroeder (1976) described consecutive histopathological features of gingivitis under the titles of initial, early, established, and advanced lesions.

The initial lesion: The initial lesion, develops within 1-2 days of cessation of plaque control. It is an acute inflammatory reaction and is characterised by the migration of neutrophils and monocytes from surrounding nearby vessels into the connective tissue, junctional epithelium, and gingival sulcus. A proportion of perivascular collagen fibres may disappear and gingival fluid flow is increased as a result of enhanced permeability of the gingival vessels.

The early lesion: The early lesion is seen 4-7 days after plaque accumulation. It features infiltration by lymphoid cells which are mainly T cells. The number of PMNs and monocytes also continues to rise. Some fibroblasts may show early signs of degeneration. Further loss of the collagen fibre network supporting the marginal gingivae is

observed. The basal cells of the junctional epithelium begin to proliferate.

The established lesion: Although the original model of Page and Schroeder suggested that the established lesion occurs 2-3 weeks after plaque accumulation, more recent evidence indicates that the time taken for the established lesion to develop is uncertain (Brecx *et al.*, 1988). It is characterised by a cellular infiltrate dominated by plasma cells and B lymphocytes, continuing loss of connective tissue substance noted in the early lesion, and proliferation and lateral extension of the junctional epithelium. Gingivitis at this stage is still reversible if oral hygiene is re-instituted, otherwise, it may either remain unaltered for extended periods of time or progress to an advanced lesion, i.e., periodontitis.

1.2.3.2 Periodontitis

Periodontitis is an inflammatory disease of the attachment apparatus. Its features are characterised by dominant plasma cell infiltration, bone resorption, destruction of periodontal ligament fibres and apical migration of the junctional epithelium. Progression of periodontitis may ultimately result in tooth loss. Interactions between the host defense system and the pathogenic microflora are generally considered to be the key features of the pathogenesis of the periodontitis.

1.2.3.3 Bacterial plaque

Bacterial plaque may be defined as an aggregation of bacteria colonizing the tooth surface (Kelstrup and Theilade, 1974). The bacteria are attached together or to the tooth surface either directly or via a matrix of carbohydrates and proteins, called the inter-microbial matrix. A few minutes after exposure of the cleaned tooth surface to saliva, an acellullar and homogenous pellicle of salivary glycoproteins called "acquired pellicle" is formed which glues the bacteria to the tooth surface. Early plaque is dominated by Gram positive coccal organisms and short rods. Gradually the number of Gram negative coccal organisms and rods increases and after a few days filamentous micro-organisms appear followed by spirilla and spirochetes (Theilade *et al.*, 1966). If the plaque remains undisturbed, as it becomes thicker, the environment shifts to one which is more hospitable to anaerobic micro-organisms especially in the deeper layers next to the tooth surface (Ritz, 1969). In advanced periodontitis, the cultivable flora is comprised mainly of anaerobic micro-organisms and the proportion of spirochetes increases with increasing pocket depth (Listgarten and Levin, 1981).

1.3 The role of bacteria in the aetiology of periodontal disease

The relationship between plaque and periodontal disease is now universally accepted. Löe, Theilade and Jensen

(1965), Theilade *et al.* (1966), and Jensen *et al.* (1968) showed that after refraining from oral hygiene measures, dental plaque develops, closely followed by inflammation of the gingiva. They observed that after plaque removal the gingivitis disappeared within a few days.

There are many mechanisms by which the bacteria may contribute, directly, or indirectly to the tissue destruction. It has been suggested that the bacteria may invade the marginal periodontal tissues (Frank, 1980; Saglie *et al.*, 1982a,b; Gillett and Johnston, 1982; Saglie, Carranza and Newman, 1985; Christersson et al., 1987; Saglie *et al.*, 1988; Lamont, 1992; Duncan *et al.*, 1993). However, scepticism has emerged because of the possibility of artefacts during the preparation of tissues (Liakoni, Barber and Newman 1987). Nevertheless, it appears from the results of controlled *in vitro* studies (Sandros, Papapanou and Dahlen, 1993; Sandros *et al.*, 1994; Papapanou *et al.*, 1994) that some bacteria have the ability to invade host cells. The bacteria may also induce damage through release of their toxic products. Sulphides, thiols, ammonia, and indole are some toxic metabolites of the inhabitants of the pocket flora. Bacteria may also release products which adversely affect the homeostasis of the periodontium either by suppressing the host response or by inducing immunologic reactions beyond the protective role of the host which could result in tissue destruction. Some bacteria can degrade IgA and

IgG (Kilian, 1981). Some Bacteroides may secrete a surface coating which makes them resistant to phagocytosis (Sundqvist *et al.*, 1982). Proteinases may be released by bacteria (Fujimura and Nakamura, 1981). *A.a.* produces a leukotoxin which kills human PMNs and monocytes (Tsai *et al.*, 1979; Baehni *et al.*, 1979). Van Dyke *et al.* (1982) suggested that several species release peptides that can bind to and block the receptors for chemoattractant molecules, found on the surface of phagocytic cells. Lipopolysacharide (LPS) of Gram negative bacteria can induce bone resorption and activate the complement cascade, in addition to its inhibitory effect on fibroblasts.

1.4 Host mediated tissue damage

The discovery of several specific regulatory mediators was a breakthrough in the understanding of host response mechanisms. Cytokines, chemokines, growth factors, prostaglandins, leukotrienes, thromboxanes, anaphylatoxins, kinins, and biologically active amines all play roles in the induction, amplification, persistence, control and resolution of localized inflammatory immune responses. The stimulation by the bacteria and their products, of host cells which release cytokines, particularly interleukin-1 and tumour necrosis factor-alpha, may trigger the activation of tissue destructive mechanisms by the host. There are at least 4 distinct mechanisms which are involved in the remodelling of the

tissue stromal architecture. These are the osteoclast pathway, the matrix metalloproteinase pathway, the plasminogen-dependent pathway, and the intracellular lysosomal dependent phagocytic pathway (Birkedal-Hansen, 1993). While the first pathway is involved in the degradation of the mineralised matrices, the matrix component of the connective tissue is the target of the second and third pathways and finally the fourth pathway is responsible for the intra-cellular digestion and disposition of larger fragments such as collagen fibrils (Meikle, Heath and Reynolds, 1986).

1.5 The clinical assessment of periodontium

1.5.1 Plaque index

Plaque index is an expression of plaque accumulation on the tooth surface. The choice of plaque index for a clinical trial should be made with regard to the objective of the trial. Currently, plaque indices estimate plaque accumulation by measuring either the tooth surface area covered or the thickness of plaque in the area. One of the earliest plaque indices for the assessment of debris was introduced by Ramfjord (1959) as part of his periodontal index and was modified by Shick and Ash (1961) and is based on the surface area covered by plaque on 6 Ramfjord teeth. This index, having a range from 0 to 3, measures the plaque on the lingual and buccal surfaces and the plaque status on the proximal surfaces has only a minor contribution to the score. The simplified oral

hygiene index (OHI-S) of Greene and Vermillion (1964) assesses the debris accumulation based on the tooth surface area using a scale ranging from 0 to 3. The plaque index of Quigley and Hein (1962) scores the area covered by plaque from 0 to 5. Until the early 60's, most plaque indices assessed mainly the buccal and lingual surfaces. Furthermore, these plaque indices tended to overscore the incisal portion of the tooth at the expense of the cervical portion. When plaque is evaluated in trials of periodontal disease, the amount of plaque in close contact with the gingivae is of greater importance. Taking this into consideration, Silness and Löe introduced their plaque index in 1964. The index was described in more detail by Löe (1967). It is still one of the most widely used plaque indices in clinical studies of gingivitis and periodontitis. The index scores the thickness of plaque on the gingival area of each of the 4 surfaces of the tooth. However, because this index is disruptive (when discriminating between score 0 and 1 using the probe), the evaluation of the inter- and intra-examiner variability prior to a study is not possible. For this reason and because of the relative subjectivity of the index, only one examiner should be fully trained and used throughout the study period. The Navy Plaque Index (Elliot et al., 1972), divides the tooth surface into 9 parts 6 of which are located in the gingival third. This index and its modification (Fischman, 1986), although

putting more emphasis on the gingival part, seem to be difficult for the examiner to learn and remember.

The use of a plaque index in a clinical trial evaluating the efficacy of clinical modalities for the treatment of periodontal disease can provide some evidence of the effectiveness of patients' plaque control. Obviously, the quality of patients' home care will affect the response to the therapy and should be monitored and kept at a high standard during the study period and more importantly should be balanced between the treatment groups. However, since brushing by the patients shortly before the clinical measurements may invalidate the plaque score data, to draw conclusions about the performance of patients in their plaque control, plaque index should be supplemented with a gingival index or a bleeding index. These latter indices could fairly, at least in gingivitis with little periodontal destruction, act as evidence of recent plaque exposure.

1.5.2 Gingival index

Once gingivitis and periodontitis were thought to be different stages of the same disease. Naturally, the indices designed to assess the disease covered both entities in their scales. Among these were the PMA (P:papillary, M:marginal & A:attached) Index (Schour and Massler, 1947), Russell's Periodontal Index (Russell, 1956), the Periodontal Disease Index (Ramfjord, 1959), the

Gingiva-Bone Count Index (Dunning and Leach, 1960) and the Navy Periodontal Disease Index (Elliot *et al.*, 1972). Most of these indices were developed for epidemiological studies and the tooth rather than the site was the unit of examination and for each subject one value would be calculated based on the score obtained from all or some teeth. However, concurrent with the evolving of the knowledge of periodontal disease and the increasing interest in the aetiology and pathogenesis of periodontal destruction, the following changes occurred in more recent gingival indices.

1) By differentiating gingivitis from periodontitis, most authors used gingival indices as a means of assessing the inflammation in the gingivae alone. The severity of periodontal destruction was assessed by measuring pocket depth and bone loss.

2) The gingival unit was defined as a localized area of the gingiva adjacent to a certain aspect of the tooth (e.g. mesiobuccal or buccal site) rather than the entire gingiva surrounding the tooth. As a result, more emphasis was put on the severity of the gingival inflammation at a site rather than its superficial extent and location. The gingival index of Löe and Silness (1963) was able to measure the severity of gingival inflammation at a site on the basis of both visual examination and bleeding on probing. The scores ranged from 0 to 3. Among the other

gingival indices were: the Sulcular Bleeding Index (Muhlemann and Mazor, 1958; Muhlemann and Son, 1971), the Gingival Bleeding Index (Ainamo and Bay, 1975) and the papillary bleeding index (Muhlemann, 1977; Barnett, Ciancio and Mather, 1980).

3) Up until the mid 80's, most indices used the bleeding on probing as a part of or the entire criteria of scoring the gingival inflammation. With growing interest in the assessment of crevicular fluid components, it became necessary to develop indices which would allow the assessment of gingival inflammation without inducing any trauma to the gingival tissue. The stimulation of the gingival tissue by the probe could transiently produce an increased flow of GCF and obscure the profile of various biochemical components. Therefore, the modification of previous indices was introduced so that the gingival examination could be assessed by mere visual examination. The gingival index of Löe and Silness (1963) was modified by Lobene *et al.* (1986). In addition to elimination of the bleeding component, an additional score was incorporated in the index and the criteria for mild and moderate inflammation was redefined. Relying only on the visual signs of inflammation does not reduce the ability of an index to detect gingivitis. Morphometric studies indicated that the presence of visual signs of gingivitis alone significantly correlated with the presence of

inflammatory infiltrate in the gingival connective tissue (Greenstein, Caton and Polson, 1981).

1.5.3 Gingival crevicular fluid (GCF) flow

The volume of Gingival crevicular fluid has been shown to correlate with the degree of inflammation in the gingival tissue (Brill, 1960; Mann, 1963; Egelberg, 1964; Löe and Holm-Pedersen, 1965; Egelberg and Attström, 1973; Borden, Golub and Kleinberg, 1977). One non-invasive method of collection of GCF includes the insertion of filter paper strips at the orifice of the pocket. The Periotron 6000 measures the conductivity of the wetted paper strip which is in turn a function of the volume of fluid absorbed onto the paper. This method, originally developed to differentiate healthy gingiva, gingivitis and periodontitis, has been reported to correlate with other clinical methods of measuring the gingival inflammation (Suppipat, Johansen and Gjermo, 1977; Garnick, Pearson and Harrel, 1979).

1.5.4 Bleeding on probing

In the evaluation of periodontal destruction, the Modified Gingival Index alone is of limited value because it tells little about the inflammatory status of the deeper portions of the periodontium. Hence, the use of a bleeding index in addition to the Modified Gingival Index has been recommended (Lobene *et al.*, 1989). A number of bleeding indices were cited in section 1.5.2. Some

bleeding indices rely on stimulation of the superficial portion of the periodontium. Among these is the Bleeding Points Index (Lenox and Kopczyk, 1973). These indices were introduced to monitor the gingival health and oral hygiene performance of the patient and are of limited value in the assessment of periodontal destruction. Hence, in the evaluation of periodontitis, the probe should be aimed at the base of the pocket. Bleeding on probing may be attributed to alteration of blood vessel walls, weakening and ulceration of pocket epithelium, and reduction in the collagen fibres around the blood vessels (Greenstein, 1984). However, the occurrence of bleeding after probing into the base of the pocket is not necessarily indicative of inflammation in this area.

Preferably a standardised probing force should be used when probing to assess bleeding since the tendency towards bleeding increases with increasing probing force (Freed, Gapper and Kalkwarf, 1983; van der Velden, 1980; Caton, Proyne and Polson, 1982; Proye, Caton and Polson, 1982; Lang *et al.*, 1991). The Periodontal Pocket Bleeding Index (van der Velden, 1979) uses a constant force of 0.75 N and dichotomised scores are given to each site if it shows bleeding within 30sec of probing. 30 seconds seems a sufficient length of time to allow the micro-haemorrhage which occurs at the base of the pocket to show itself at the orifice of the pocket. Lang *et al.* (1991) and Karayiannis *et al.* (1992) studied the effect of varying

probing forces on bleeding scores in healthy gingiva and observed that with a probe tip diameter of 0.40 mm, minimal bleeding was found using a probing force of 0.25 N (about 24 g). It seems that bleeding provoked by forces higher than 0.25 N may be caused by trauma to healthy blood vessels rather than the rupture of fragile vessels in inflamed tissues. If probing forces above 0.25 N are preferred, bleeding scores of clinically healthy gingiva may not approach 0%. In addition, the reduction of bleeding on probing scores after treatment, relative to baseline scores, have been found greatest for the lower probing forces (van der Velden, 1980; Caton *et al.*, 1982; Proye *et al.*, 1982).

There has been debate in the literature regarding the value of bleeding on probing. Janssen, Faber and van Palenstein Helderman (1986) reported limited reproducibility for bleeding on probing. For clinical purposes, therefore, bleeding on probing alone may not be reliable on an individual site basis and should be interpreted with caution. Nevertheless, despite these limitations changes of bleeding scores for pooled sites in a patient seem to be an indicator of improvement in periodontal condition as a result of the treatment. Gingivae that do not bleed on probing have been shown to contain a smaller inflammatory infiltrate and denser collagenous connective tissue, than those that bleed (Polson, Greenstein and Caton, 1981; Cooper, Caton and

Polson, 1983; Davenport, Simpson and Hassell, 1982; Greenstein, Caton and Polson, 1981; Engelberger, Hefti and Kallenberger, 1983). These studies indicated that bleeding on probing demonstrate a substantial deviation from health. Generally, the absence of bleeding on probing in a site that previously bled may be interpreted as improvement in periodontal health and an absence of disease (Greenstein, 1984; Lang *et al.*, 1990).

1.5.5 Probing depth and probing attachment level

Periodontal probing has been one of the most fundamental methods of periodontal examination. The simplicity of this method has allowed clinicians to evaluate the presence of a pathologically deepened sulcus. The reliability of periodontal probing has been extensively studied. All published studies on evaluation of periodontal probing have dealt with one or more measures of the quality of this diagnostic tool; resolution or precision, validity or accuracy, and repeatability (Jeffcoat and Reddy, 1991a). Resolution is related to the smallest increment which the probe can measure and is dependent on the design of the probe. Higher resolution *per se* is not necessarily synonymous with higher reproducibility. However, if an examiner could perform highly reproducible probings, the higher resolution would play a role by resulting in both more precise measurements and improved variability. Accuracy is closeness of the probe reading to the parameter which the probe is meant to

measure i.e. the distance from the gingival margin or cemento-enamel junction (CEJ) to the most coronal transeptal connective tissue fibres. This may depend on factors related to probe design such as probing force and the probe tip diameter, as well as factors related to the status of the periodontal tissues such as degree of inflammation. The last factor i.e. reproducibility is related to a great extent to the operator skill and careful characterisation of the site to be probed. Furthermore, the factors related to the probe design and the tissue status play a role in the reproducibility of the measurements. It has been consistently reported that a significant relationship exists between the degree of inflammation and the level of probe penetration (Armitage, Svanberg and Löe, 1977; Robinson and Vitek, 1979; Jansen, Pilot and Corba, 1981; Magnusson and Listgarten, 1980; Caton *et al.*, 1981; Fowler *et al.*, 1982; Garnick *et al.* 1989).

The measurement of true connective tissue attachment is a critical factor when evaluating the outcome of regenerative procedures from which a coronal re-growth of connective tissue attachment is expected. Moreover in monitoring the disease progression in periodontitis sites, the major interest is focused on the apical displacement of connective tissue attachment levels in relation to a reference point such as CEJ. However, after standard procedures such as root planing or any procedure which

results in the resolution of inflammation, the relative attachment change over a time period is mainly a reflection of increase in density of connective tissue fibres and formation of a long junctional epithelium (clinical attachment gain). While the observed changes in probing attachment may be substantial, the true regeneration of transeptal fibres is usually small. Even if a probing technique is capable of predictably measuring the connective tissue attachment levels before treatment, it will underestimate the attachment levels after therapy. Therefore, the consistent and accurate estimation of true connective tissue attachment during the assessment of procedures which result in clinical attachment gain with little true attachment gain, is neither achievable nor practically important and reflective of treatment success.

In addition to the degree of inflammation, there are a number of factors which play a role in the reproducibility of the probing. These variables include diameter of the probe tip (Keagle *et al.*, 1989), angulation of the probe to the root surface (Karim, Birek & McCulloch, 1990; Watts, 1989), accuracy of probe markings (van der Velden, 1978; Winter, 1979; van der Zee, Davies and Newman, 1991), and the identification of a reference point such as CEJ in the case of attachment level measurements. The CEJ has been reported to be masked by its subgingival location or by the presence of restoration margins or by the presence of calculus in 17% of the sites (Clark et al., 1987). In

an attempt to reduce errors in the identification of the CEJ as well as errors in the placement of the probe, occlusal stents have been used and reported to be useful (Isidor, Karring and Attström, 1984a; Badersten *et al.*, 1984a; Clark *et al.*, 1987). Probing force has also been shown to be an important factor in the degree of probe penetration (van der Velden, 1979; van der Velden and Jansen, 1981; Chamberlain *et al.*, 1985 and Garnick *et al.*, 1989). Since the late 70's many investigators have used controlled force probes to improve the reliability of the probing measurements (van der Velden and de Vries, 1978; Caton *et al.*, 1982; Proye *et al.*, 1982). While the superiority of pressure controlled probes over manual probing is not universally accepted (van der Velden and de Vries, 1980; Badersten, *et al.*, 1984a; Simons and Watts, 1987), most data indicates that these probes are useful instruments in reducing the measurement error. Freed *et al.* (1983) reported that the force of probing may vary from 5 to 125 g among clinicians. Similar findings were reported by Hassel, Germann and Saxer (1973). Moreover, it was reported that distal sites and posterior teeth are probed at higher forces than mesial sites and anterior teeth, when conventional probes are used. van der Velden (1979) evaluated the position of the probe tip in relation to the true connective tissue attachment level at different probing forces. It was reported that with increasing probing force, the probe penetration increased and using a probe tip of 0.63 mm diameter, the probing

force of 0.75 N produced the best indication of true attachment levels. However, in van der Velden's study the probing was performed on treated periodontitis sites. Since the diseased sites are of the most interest to clinicians, it would be more realistic if the diseased sites had been evaluated for optimal probing force. Walsh and Saxby (1989) reported 100% agreement within 1 millimetre between pairs of measurements when a pressure sensitive probe with a force of 0.25 N was used. The corresponding value for the manual probe was 96%. They also reported that the use of a constant force probe improved the inter-examiner variability but only one of the examiners benefited from the pressure probe in improving the intra-examiner variability. Similar findings indicative of a lack of benefit of pressure probes on the intra-examiner variability were reported by Abbas *et al.* (1982). These data indicate that some operators can probe more reproducibly than others and that operator skill is an important source of variation. van der Velden and de Vries (1980) reported no advantage for the pressure probe using a force of 0.75 N in terms of variability. Mombelli, Mukle and Frigg (1992) reported that while the amount of measured attachment gain after therapy is higher at low-force probings, the higher force probings were more reproducible. These 2 factors i.e., the mean difference between pre- and post-therapy and measurement error are 2 major determinants which dictate whether a treatment outcome is statistically significant

or not. It appears that at each probing force, one of these factors could be improved at the expense of the other one. In this context, it is worth mentioning the study of Proye *et al.* (1982), who reported that controlled force probing using 25 g or 50 g was capable of detecting a significant attachment gain at 3 and 4 weeks post-therapy, whereas manual probing at the same sites failed to detect the attachment gain at the 4 week time point.

Deeper measurements by the conventional probes as compared to pressure sensitive probes have been reported by many investigators (Proye *et al.* 1982, Caton *et al.* 1982, Osborn *et al.* 1990)

1.5.5.1 Electronic pressure sensitive probes

A pressure sensitive probe which automatically detects the CEJ was described by Jeffcoat and Reddy. Using this probe a standard deviation of less than 0.2 mm was found for individual sites (Jeffcoat and Reddy, 1991b). An electronic pressure sensitive probe, the Florida Probe, which measures pocket depth and attachment level to the nearest 0.1 to 0.2 mm was developed by Gibbs *et al.* (1988) and has a reported reproducibility of 0.58 mm and 0.28 mm for probing depth and attachment level (Stent probe) respectively (Gibbs *et al.*, 1988; Clark, Yang and Magnusson, 1992). The Florida Probe is equipped with different handpieces which allow pocket depth measurement as well as the measurement of attachment level both from a

custom-made stent and the occlusal surface (Magnusson *et al.*, 1988a; Osborn *et al.*, 1990). Magnusson *et al.*, (1988a) reported that both intra-examiner and inter-examiner variability of probing depth measurements were significantly reduced when the controlled force Florida Probe was used instead of a conventional probe. The standard deviation of duplicate measurements of attachment levels decreased when the Florida Probe was used (Magnusson *et al.* 1988b) as compared to manual probes. This resulted in a subject threshold as low as 0.60 mm for severe periodontitis sites. It was concluded that the changes in attachment levels could be detected earlier if the Florida Probe is used instead of manual probes. Osborn *et al.*, (1990) compared the reproducibility of the conventional probe with the Florida probe with a force of 20 g. It was reported that intra- and inter-examiner variability of measurements of probing depth and attachment levels using a single pass of the Florida probe did not offer any advantage over conventional probing. However, duplicate measurements using the Florida probe showed less variability compared to the single measurement by conventional probes. This was especially true for attachment level measurements. Although the improvement in the reliability of probing as a result of duplicate measurements is a statistical phenomenon, it could be particularly useful when blind measurements are obtained. The standard deviation of differences in 2 consecutive measurements is reduced by a factor of the square root of

2 if duplicate rather than single measurements are taken. However, obtaining duplicate measurements using conventional probing can not be relied upon unless 2 separate visits are used to take the readings. Since the examiner reads the measurement, he/she probably remembers the first reading, when the second measurement is being obtained. This will result in a bias in the second reading making it artificially close or equal to the first reading. This problem is overcome when obtaining measurements using the Florida probe, since the reading appears on a computer screen and recorded by an assistant and no graduation exists on the probe tip, the examiner remains blind to the actual reading and the possibility of bias in the probing is eliminated. Marks *et al.* (1991) reported a higher reproducibility for the 'Stent Florida Probe' as compared to the 'Disk Florida Probe'. However, only sites with minimal periodontal disease were included in the study. Another electronic pressure sensitive probe, the Toronto Probe, was first described by Birek, McCulloch and Hardy (1987) and later modified by Karim, *et al.* (1990). It measures attachment level to the nearest 0.1 mm under controlled angulation with a reported reproducibility of 0.46 mm. An electronic pressure sensitive probe, Inter-probe, uses disposable flexible plastic tips at a force of 15 g, has a resolution of 0.5 mm and measures only the pocket depth. The reproducibility of this probe was reported to be 0.8 mm (Goodson and Kondon, 1988) and did not offer any advantage

over the manual probes (Wang *et al.*, 1995). Possibly, the flexibility of the tip and excessively low force of probing are responsible for the poor reproducibility of this probe. It is worth mentioning that the reproducibility of manual probing for attachment levels has been reported as 0.84 mm (Haffajee, Socransky and Goodson, 1983b). The quoted reproducibility of the mentioned probes was dependent on figures reported by investigators in separate studies, which are obviously not comparable because different operators and patients were used for each study.

In conclusion, it appears that: a) most pressure sensitive probes offer advantages over manual probes; b) due to large operator-dependent measurement variations, a single operator should be trained and used throughout longitudinal studies if possible; c) careful localisation of the sites, through exact characterisation and possibly the use of occlusal stents are essential for reduction of measurement errors; and d) duplicate recordings may be useful in further reducing the variability. Despite all the factors which limit the interpretation of probing data, probing pocket depth and probing attachment level, still comprise the major outcome variables and the periodontal probe still remains one of the most reliable tools in the hands of therapists.

1.6 Treatment strategies in periodontal therapy

The methods of treating periodontal disease can be categorised into 4 basic groups; 1) resective therapy, 2) mechanical therapy, 3) regenerative therapy, and 4) antimicrobial therapy.

1.6.1 Resective therapy

Briefly, resective therapy includes the surgical elimination of the periodontal pocket. The proponents of this approach believe that the total elimination of the periodontal pocket and establishment of a normal anatomy, but at a reduced height is the only means of arresting the progression of the disease. In this approach, the marginal bone may be resected to re-establish normal contour (Page, 1993). The pocket wall is eliminated either by excision or apical re-positioning of the flap.

1.6.2 Mechanical therapy

This approach includes the non-specific elimination of bacterial plaque, calculus and other plaque retentive factors from the pocket by means of mechanical disruption. A surgical flap may be reflected only to aid visual access (Ramfjord and Nissle, 1974). In this strategy the gold standard is not zero pocket depth but rather conversion of infected pockets to healthy pockets, maintained free of pathogenic bacteria. Since the late 1970's many longitudinal studies have been conducted to compare the ability of mechanical therapy to other treatment

modalities. The work of several investigators has demonstrated that while pocket elimination may favour a reduction in pocket depth, scaling and root planing or modified Widman surgery favour a gain in clinical attachment levels (Ramfjord *et al.*, 1975; Knowles *et al.*, 1979; Knowles *et al.*, 1980). In a 5 year clinical study, Hill *et al.* (1981) and Ramfjord *et al.* (1987) showed that scaling and root planing and curettage produced more attachment gain than pocket elimination surgery in moderately deep pockets while no difference was found between treatments in very deep pockets. Moreover, in the shallow sites the surgical elimination technique resulted in the largest attachment loss. Kaldahl *et al.* (1988) reported that clinical attachment gain is more favourable after the modified Widman flap procedure or scaling and root planing when compared to pocket elimination surgery or supragingival scaling. The results of studies by Isidor, Karring and Attström (1984b), Lindhe *et al.* (1984), and Isidor and Karring (1986) indicated that there was no difference between scaling and root planing and the modified Widman technique. In summary, longitudinal trials demonstrated that scaling and root planing and surgical procedures were equally effective in arresting the progression of periodontal disease. It appears that the major determinant of the periodontal therapy is thorough root debridement and good oral hygiene.

1.6.2.1 The practical limitation of the traditional mechanical therapy

Having mentioned the efficacy of mechanical therapy, it should be stated that there are some factors which sometimes limit the practicality of traditional mechanical therapy. Scaling and root planing is very time consuming. In the study of Ramfjord *et al.* (1987) scaling and root planing amounted to 5 to 8 hours by a hygienist for the whole mouth plus 1.5 hours per quadrant by a periodontist. In other studies the root planing required 6 to 8 hours per patient (Pihlstrom, Ortiz-Campos and McHugh 1981; Lindhe *et al.*, 1984). The other limiting obstacle is the skill of the operator. There is a considerable variability among clinicians in their ability to perform root debridement effectively. (Badersten, Nilvéus and Egelberg, 1985; Eaton, Kieser and Davies, 1985; Brayer *et al.*, 1989). A thorough root debridement can be painful for patients. Dentinal sensitivity may occur which is often exacerbated by structural wear of the tooth due to long term maintenance therapy with root planing at 3 monthly intervals. If the root debridement necessitates flap reflection, then the complications encountered during or after the procedure should also be added to the list of limitations of mechanical therapy. Finally, some sites respond less favourably than others to mechanical therapy (Hirschfeld and Wasserman, 1978; Loos *et al.*, 1989). However, it is not clear whether the failure of the treatment is due to incomplete root debridement and/or

inadequate home care or a deficient host response, or whether a peculiarly persistent micro-organism(s) is responsible. Whatever the aetiology of persistent periodontitis, the occasional lack of a predictable response to treatment, the time obstacle, the variation in operator skills, and the short term and long term patient discomfort, have encouraged the clinical scientists to seek other treatment approaches to establish periodontal health.

1.6.3 Alternatives or adjuncts to root planing

1.6.3.1 Regenerative therapy

Since Nyman *et al.* (1982a,b) demonstrated the possibility of regeneration of periodontal tissues on previously diseased surfaces using a physical barrier, there have been many efforts to develop practical and predictable techniques and materials to achieve this goal. It is not the scope of this thesis to review the studies on the regeneration potential of bone and periodontal ligament using different graft materials, membrane placement, surgical techniques or chemical modification of the root surface. While much research is going on and some advances have been made, it seems that a practical, predictable and cost-effective regenerative technique is yet to be developed.

1.6.3.2 Antimicrobial therapy

Shortly after bacterial plaque was discovered as the major aetiological factor in periodontal disease (Löe *et al.*, 1965), antimicrobial agents were demonstrated to produce qualitative and quantitative changes in plaque (Löe *et al.*, 1967, Jensen *et al.*, 1968). Since then, the debate has been opened as to whether the antimicrobials could be used as an adjunctive/sole treatment for periodontal disease and several investigators tested the efficacy of antibiotics for this purpose (reviewed by Slots and Rams 1990, Gordon and Walker 1993, Greenstein 1993, Kornman 1993, Seymour and Heasman 1995a,b).

1.7 A review of systemic antibacterial therapy for the treatment of periodontal disease

1.7.1 Introduction

A single micro-organism or groups of micro-organisms have been reported as having a strong association with periodontal disease. If a micro-organism is proved to be both a periodontal pathogen and exogenous, then its specific elimination from the pocket is necessary. As yet no such direct causative relationship has been recognised. In addition, of the suspected periodontal pathogenic bacteria, no single species has been linked with diseased sites which is not also commonly isolated in non-diseased sites. Non-selective cultures have revealed that the subgingival ecosystem, is comprised of more than 300 species, and has a complex and variable nature. Until the

many ongoing efforts elucidate this problem, the non-specific elimination of the whole mass of the microbial plaque by means of mechanical instrumentation remains the main tool in the hands of the therapist. Nevertheless, the increasing evidence for the association of specific species with some forms of periodontal disease, has increased the interest in the use of antibacterial drugs for elimination of these organisms. Many systemic and local forms of antimicrobial agents were developed to eliminate suspected pathogens from the subgingival ecosystem. Many studies have been conducted by clinical researchers to examine whether these agents could be effective in arresting the progression of periodontal disease either as the sole treatment or as an adjunct to mechanical therapy. In the following sections, the background information concerning the use of antibacterial agents in the treatment of periodontal disease will be reviewed and discussed.

1.7.2 Tetracyclines

To date, tetracyclines are the only antibiotics that following systemic administration achieve gingival fluid levels higher than serum levels. Multiple oral doses of tetracycline, doxycycline and minocycline produce GCF concentrations of 2-5 times serum levels (Gordon *et al.*, 1981; Ciancio, Mather and McMullen 1980; Ciancio *et al.*, 1982; Pascale *et al.*, 1986). Dental tissues can act as a reservoir which prolong the substantivity of the drug

(Baker *et al.*, 1983; Bjorvatn, Skaug and Selvig, 1985). Most micro-organisms considered periodontal pathogens are susceptible to the tetracycline concentration in GCF after systemic administration (Walker *et al.* 1981a; Walker, Gordon and Socransky 1983; Walker *et al.*, 1985; Baker *et al.*, 1985). Lindhe and Liljenberg (1984) reported that a combination of surgery and 1 g/day tetracycline for 2 weeks was successful in the treatment of localized juvenile periodontitis (LJP) and adult periodontitis patients. Slots and Rosling (1983) reported that whilst scaling and root planing was ineffective in eliminating *A.atinomycetemcomitans* (*A.a.*) from periodontal pockets of patients with LJP, tetracycline administered in 250 mg doses 4 times a day for 14 days was successful in eliminating *A.a.* from half of the treated sites, until 38 weeks post-therapy. The authors concluded that the tetracycline should be continued for 3 weeks. Kornman and Robertson (1985) reported that in patients with LJP, scaling plus tetracycline given in 250 mg doses of 4 times a day for 4 weeks, was more successful than scaling alone in sites in which *A.a.* was detected. Mandell *et al.*, (1986) used 100 mg doxycycline a day for 2 weeks. It was reported that until 10 months after therapy the combination of doxycycline and scaling was more successfull in arresting the attachment loss than doxycycline alone. Mandell and Socransky (1988) reported that the combination of surgery and doxycycline therapy of 100 mg daily for 14 days was successful in elimination of

A.a. from the sites of 6 out of 8 patients with LJP after 1 year. Although these studies suggest a beneficial role for tetracyclines in the treatment of LJP, there are reports in the literature that indicate that mechanical therapy alone is successful in treating LJP lesions (Waerhaug, 1977; Christersson *et al.*, 1985; Saxen *et al.*, 1986).

There are a number of reports indicative of the efficacy of the tetracycline family as adjuncts to mechanical therapy for adult periodontitis (Lindhe, Liljenberg and Adielsson, 1983; Haffajee, Dzink and Socransky, 1988; Papli and Lewis, 1989). Furthermore, some investigators reported success after the use of tetracyclines for refractory periodontitis (Slots *et al.*, 1979; Rams and Keys, 1983; Rams *et al.*, 1985; McCulloch *et al.*, 1990). On the other hand, Listgarten, Lindhe and Hellden (1978), Hellden, Listgarten and Lindhe (1979) and Scopp *et al.* (1980) found no advantage in using adjunctive tetracycline or tetracycline alone. Ciancio *et al.* (1982) found that adjunctive minocycline brought no advantage over scaling alone in reducing the pocket depth although the gingival inflammation was improved more favourably after the combination therapy. In a series of animal studies, Williams *et al.* (1981a), and Williams, Jeffcoat and Goldhaber (1982) reported that long-term treatment with low dosage tetracycline reduced the rate of bone loss in the experimental group, although their data indicated that

18 months after the start of treatment, a trend towards baseline was observed in the rate of bone loss. Kornman and Karl (1982) reported that 250 mg tetracycline per day was effective in treating refractory periodontitis lesions. The tetracycline reduced the suspected periodontal pathogens, although the levels of tetracycline resistant bacteria were increased. However, following the cessation of tetracycline the disease recurred. Lindhe, Liljenberg and Adielsson (1983) reported that long term tetracycline was more beneficial than scaling alone in reducing pocket depth, attachment loss, and bleeding on probing. Tetracycline at a dosage of 250 mg 4 times a day was given for 2 weeks, followed by 250 mg once a day for the rest of the 12 month period of the study. While the published data regarding the effect of long term and low dose tetracycline is not yet sufficient to prove its efficacy with certainty, this approach should be looked at with great care because of the risk of bacterial resistance which is relevant to the long-term and low-dosage use of wide spectrum antibiotics such as the tetracyclines. Animal studies indicated that long-term use of tetracycline changed the subgingival microflora to one more resistant to tetracycline (Williams *et al.*, 1979; Williams *et al.*, 1981b). Although currently, tetracyclines are not usually used in medicine for dangerous and life threatening infections and therefore tetracycline resistance would not be as important, the plasmid associated bacterial resistance, if developed to

one antibiotic e.g. tetracycline, will usually also promote resistance to a large number of similar antibiotics. In periodontal pocket, antibiotic resistance may occur in *A.a.* and *C.consicus* upon continued exposure to subinhibitory concentrations of the antibiotic (O'Connor, Newman and Wilson, 1990). When a systemic antibiotic such as tetracycline is used, theoretically, a systemic serum level, effective against susceptible strains is achieved at the site of infection. However, it could be argued that some bacteria from the pocket (such as *Eikenella Corrodens,* and *Selenomonas Sputigena*) may not be entirely inhibited by systemic levels of tetracycline, and may develop and transmit tetracycline resistance to other species.

On the other hand, if a local antibiotic is used, it could be assumed that the periphery of the treated area may receive an antibiotic concentrations below the effective levels. Unless the topical treatment can provide a high concentration of antibiotic, which covers the entire infected area, the chance of development of antibiotic resistance exists. In case of periodontal therapy using topical agents, this may occur if the topical agent fails to reach the most apical plaque front or it reaches there only at sublethal concentrations. Furthermore, if the topical treatment is going to be repeated, the substantivity of the system should sustain an antibiotic level above the inhibitory levels during the interval

between two applications. It has been reported that both locally delivered and systemically administered minocycline in periodontal tissues, produce a transient increase in the proportion of minocycline resistant bacteria. However, for both types of treatment, after a few weeks these proportions returned to pre-treatment values (Preus *et al*. 1995).

Some important side effects of tetracycline include discoloration of developing teeth in children, nausea, vomiting, diarrhoea, erythema, dizziness and vertigo (minocycline), headache and visual disturbances (may be indicative of benign intracranial hypertension), severe exfoliative rashes (minocycline), liver damage, photosensitivity and pseudomembranous colitis. Tetracyclines should not be prescribed for children under 12 years of age because of the risk of tetracycline staining of the teeth. Other contra-indications include severe renal impairment (only tetracycline), pregnancy and systemic lupus erythematous and porphyria (doxycycline). In the case of breast-feeding, hepatic and severe renal impairment, tetracycline should be administered cautiously (British National Formulary, 1994). The absorption of tetracyclines is inhibited by a number of drugs such as antacids, angiotensin-converting enzyme inhibitors, ulcer healing drugs, zinc salts and iron salts. In the presence of tetracyclines the effect of cyclosporins and anticoagulants are increased. On the other hand, the

efficacy of oral contraceptives and antiepileptic drugs are reduced if used concurrently with tetracyclines (British National Formulary, 1994).

1.7.3 Metronidazole

Metronidazole achieves a gingival crevicular fluid concentration of 14 μg/ml following multiple oral doses Giedrys-Leeper, Selipsky and Williams (1985). This level has been reported to be inhibitory for anaerobic periodontal micro-organisms *in vitro* (Sutter, Jones and Ghoniem, 1983; Baker *et al.*, 1985; Walker *et al.*, 1985; Newman *et al.*, 1979). Hydroxy metabolites of metronidazole have been reported to increase its efficacy against facultative organisms *in vivo* (Jousimies-Somer *et al.*, 1988). However, the presence of *Fusobacterium nucleatum* was reported to protect other micro-organisms against this antibiotic (Lacroix and Mayrand, 1989). The inhibition of *A.a.* and *Capnocytophaga* spp. and *Eubacterium* spp. and *Eikenella corrodens* by metronidazole requires higher concentrations than those achieved in GCF. Metronidazole has been reported to be successful in the treatment of acute ulcerative gingivitis (Duckworth *et al.*, 1966). However, the reports regarding its efficacy on adult periodontitis are equivocal. Combination of surgery with metronidazole (200 mg 4 times a day, for 7 days) was reported to have no clinical and microbiological benefit over surgery alone (Mahmood and Dolby, 1987). Despite a short lived benefit, there was no long lasting

advantage of using systemic metronidazole in periodontal patients (Joyston-Bechal, Smales and Duckworth, 1984 and 1986). Clark *et al.*, (1983) found no advantage in the use of adjunctive metronidazole in the treatment of mentally retarded periodontal patients. Sterry, Langeroudi and Dolby (1985), Giedrys-Leeper, Selipsky and Williams (1985), Jenkins *et al.* (1989) and Walsh *et al.* (1986) also reported similar negative results. On the other hand, some authors reported that systemic metronidazole could be beneficial as an adjunct (Loesche *et al.*, 1981; Lundström, Johansson and Hamp, 1984; Loesche *et al.*, 1984) or the sole treatment (Watts, Palmer and Floyd 1986; van Oosten, Mikx and Renggli, 1987) of periodontal disease. The combination of metronidazole with spiramycin has been reported as an effective adjunct to root planing (Chin Quee *et al.*, 1987).

Important side effects of metronidazole include nausea, vomiting, unpleasant taste, gastero-intestinal disturbances, rashes, urticaria and angioedema, drowsiness (rarely), headache, dizziness, ataxia, darkening of urine, peripheral neuropathy (in prolonged and intensive treatment), transient epileptiform seizures and leucopenia (British National Formulary, 1994). During pregnancy and breast-feeding it should be prescribed with caution. Disulfiram-like reactions may occur if it is used with alcohol (British National Formulary, 1994). Other drug interactions include enhancement of the effect of

anticoagulants, increased serum concentration of phenytoin, fluorouracil and lithium by metronidazole and decreased metronidazole serum concentration by cimetidine and phenobarbitone (British National Formulary, 1994).

1.7.4 Other antibiotics

Penicillins, due to the presence of beta-lactamase producing micro-organisms in the subgingival flora (Kinder, Holt and Kornman, 1986; Valdes, Lobbins and Slots, 1982) are not effective against periodontal micro-organisms *in vivo* (Walker *et al.*, 1987). *In vitro*, penicillins are effective against most subgingival bacteria but *A.a.* (Slots *et al.*, 1980). The combination of clavulanic acid and amoxicillin i.e. augmentin has been reported to have some efficacy (Magnusson *et al.*, 1989). Clavulanic acid has a beta-lactam ring which competitively binds to beta-lactamase and inactivates it. A combination of amoxycillin with metronidazole has been reported to be beneficial in the treatment of periodontal lesions and the eradication of *A.a.* in patients who did not respond to the adjunctive treatment by tetracycline (van Winkelhoff *et al.*, 1989; van Winkelhoff, Tijhof and de Graaff, 1992; Pavicic *et al.*, 1994) or minocycline (Goene *et al.*, 1990).

Clindamycin (150 mg tablets, 4 times a day) was used successfully as an adjunct in refractory periodontitis patients who had subgingival flora susceptible to clindamycin (Gordon *et al.*, 1985; Gordon *et al.*, 1990;

Walker and Gordon, 1990). This antibiotic should be used very cautiously since it may lead to the complex side effect of pseudomembranous colitis (British National Formulary, 1994).

1.7.5 Concluding remarks from the systemic antibiotic therapy

In considering the efficacy of a systemic antibiotic for periodontal lesions, 2 factors should be considered; the concentration of the antibiotic in the gingival crevicular fluid and the MIC_{90} of the antibiotic for suspected periodontal pathogens. When a local drug delivery system is to be used, an additional factor should be taken into account; substantivity. The GCF concentration of most antibiotics following systemic administration has been determined (Ciancio *et al.*, 1980; Walker *et al.*, 1981b; Gordon *et al.*, 1981; Ciancio *et al.*, 1982; Giedrys-Leppers *et al.*, 1985; Britt and Pohlod, 1986; Pascale *et al.*, 1986; van Oosten, Notten and Mikx, 1986). The MIC_{90} of most antibiotics against suspected periodontal pathogens *in vitro* has also been measured (Slots *et al.*, 1980; Sutter, Jones and Ghoniem, 1983; Walker *et al.*, 1985; Baker *et al.*, 1985; Miyake *et al.*, 1988). These studies indicate that most antibiotics appear in the GCF at higher levels than the MIC_{90} values for *P. gingivalis* and *W. recta* and *Capnocytophaga;* however, none of them are effective against *E. Corrodens* at these levels. Interestingly, even the use of a commonly used combination of 2 antibiotics,

metronidazole and amoxycillin, does not seem to inhibit this micro-organism. Only metronidazole can inhibit *Peptostreptococcus*. Metronidazole and to some extent clindamycin are effective against *Selenomonas sputigena*. *Fusobacterium* is susceptible to tetracycline, doxycycline, clindamycin and metronidazole. *P. intermedia* is susceptible to most of the commonly used antibiotics with the exception of erythromycin. Finally, the tetracycline family are the only antibiotics which inhibit *A. actinomycetemcomitans*, although the combination of metronidazole and amoxycillin has also been used successfully against this micro-organism (van Winkelhof *et al.*, 1992). It has been suggested that to inhibit a pathogen only antibiotics which achieve *in vivo* concentrations of at least 4 times their MIC values for that micro-organism should be selected (Bryan, 1982). It appears that no single antibiotic or combination of 2 antibiotics are effective against all suspected periodontal pathogens if used systemically.

1.8 Locally delivered antimicrobial systems

1.8.1 Introduction

The concept of delivering drugs directly to the site of action has attracted continuous attention from investigators in pharmaceutical research. Controlled local drug delivery systems are advantageous compared with systemic administration in a number of ways: 1) The patient's compliance plays a minor role in the treatment

outcome, if the drug is administered professionally to the site of action. 2) The total amount of the drug taken is usually reduced by several times, reducing the risk of systemic adverse effects. 3) Oral systemic drugs are absorbed in the gastrointestinal tract and the rate of absorption of some drugs may be influenced by the quality of foods taken at the time of drug administration. This may result in variations in the amount of drug uptake between and within individuals because of dietary habits. This problem can be eliminated if the drug is delivered locally to the site of action.

On the other hand, local drug delivery systems may have some potential disadvantages and limitations: 1) If the drug is carried in a polymer substance, the polymer should be neither toxic nor allergic to the host tissues. Moreover, biodegradable matrices will be transformed to one or more by-products. The biocompatibility of such by-products is a precondition for the use of any local drug system. Nevertheless, allergic reactions may occur. 2) The placement of drug release systems usually needs professional intervention and may be painful for patients and require other procedures such as local anaesthesia. 3) A number of systems such as some of those for the treatment of periodontal disease may need special care and attention by the patient, for their *in situ* retention during the treatment period. In such cases, as with

systemic drug therapy, the patient compliance is important for the achievement of therapeutic goals.

1.8.2 Terminology

The term 'local delivery' implies that the drug is used locally, but this does not necessarily mean that the drug is retained and released in a sustained manner by a controlled mechanism. Without such a mechanism, the drug would be washed rapidly out of the pocket or any specific site of action. The term 'controlled delivery' (also called 'controlled release', 'sustained release', 'timed release' and 'slow release') implies that a controlled mechanism exists to increase the bio-availability and substantivity of the drug. These systems are not necessarily applied locally. Controlled release formulations delivered systemically may be used in order to cause delay until the drug has reached the appropriate site (e.g., coated enteric aspirins formulated to dissolve in the small intestine, preventing gastric erosion), or to prolong the duration of action of a short acting drug such as nifedipine (Grahame-Smith and Aronson, 1992). The term 'topical' is normally used to describe the application of the drug to an 'exposed area' of the body, with an example being fluoride therapy in the supragingival environment (Kornman, 1993).

1.8.3 Classification

Some local delivery systems used in periodontal pockets may be a simple solution or a gel whose only task is to carry the drug into the pocket. There is no reservoir in these systems to increase the substantivity of the drug. Therefore, the drug diffuses into the tissues by its concentration gradient alone and is then cleared away. In some systems, a substance usually a polymer, may be used to act as a reservoir. Kornman (1993) classified the delivery systems used to release the drug in a sustained mode as: 1) monolithic devices; 2) polymer membranes; 3) erodible polymeric matrices, and 4) drug particles with erodible coating. In monolithic devices the drug is dispersed in a solid polymer such as acrylic resin. In membrane (reservoir) systems a core of drug is surrounded by a polymer film, e.g. dialysis tubing. In bio-erodible systems the drug is distributed in an erodible polymer which is amenable to enzymatic or hydrolytic cleavage, and as a result, the drug is released. Delivery of coated particles involves the coating of drug granules with a lipid in varying thickness. The coat thickness of each granule dictates its solution rate. Therefore a mixture of granules with coatings of varying thickness will act as a sustained release system. Similar classifications have been suggested by Langer and Peppas (1981), Brook and van Noort (1984), Needleman (1991) and Fiorellini and Paquette (1992).

1.8.4 Kinetics of the release

In membrane devices, the rate of release is determined by the Fick's law (Langer and Peppas, 1981; Parker, 1983) and is a function of the permeability of the membrane to the drug, the difference between the concentration of the drug in the receiving fluid and saturation concentration of the drug inside the membrane. As long as the drug is maintained at a saturated concentration in the device, the delivery follows zero-order kinetics, that is, the concentration of the drug is independent of the amount of the drug under process. As the core becomes unsaturated, the release falls exponentially and follows first-order kinetics. In first-order delivery, the speed of release at any given time (release rate) is dependent on the concentration of the drug inside the membrane at that time. While in zero-order delivery, the plot of the concentration in the receiving fluid against time is linear, in first-order release, the plot of the logarithm of the drug concentration against time is linear i.e., the higher the concentration inside the membrane, the faster it falls (Grahame-Smith and Aronson, 1992).

In matrix controlled systems (e.g., monolithic devices), the amount of drug released per unit area of exposed surface of the matrix after a given time depends on the diffusibility of the drug in the GCF, the solubility of the drug in the GCF, the porosity of the matrix, the

initial concentration of the drug in the matrix, and the tortuosity of the matrix (Higuchi, 1963).

In drug suspensions within an ointment base, the rate of release from the base is dependant on the concentration of the drug in the base, the solubility of the drug in the base, the diffusibility of the drug molecules in the base and the time of exposure (Higuchi, 1961). The final drug release will depend, in addition to the above factors, on the clearance of the ointment vehicle.

1.8.5 A literature review of local drug delivery systems in the treatment of periodontal disease

1.8.5.1 Hollow fibres

Goodson, Haffajee and Socransky (1979) developed a drug delivery system which consisted of hollow fibres made of cellulose acetate and filled with tetracycline. Each centimetre of fibre had a volume of 0.3 μm^3 which was filled with a 20% solution of tetracycline. When the fibres were placed in periodontal pockets for 24 hours, the half life of drug levels measured by spectrophotometry in the GCF was found to be 30 mins. In other words 95% of the drug was released over the first 2 hours. However, there was still a concentration of 15 µg/ml detectable after 24 hours. Lindhe *et al.* (1979) compared the efficacy of the system as the sole treatment with both a positive root planing alone control and a negative or untreated control. Both treatments were repeated at day

28. At each treatment the fibres were left for 2 days. The reduction in pocket depth at day 37 was 2.3 mm for root planed sites compared to 1.3 mm in fibre treated sites. A significant reduction in the proportion of motile rods and spirochetes was reported in the test group. Hollow fibres have also been investigated by another group. Addy *et al.* (1982) used hollow dialysis tubes containing 20% chlorhexidine *in vitro* and reported that 95% of the drug was released into water during the first 24 hours. After 3 days the concentration was < 1 µg/ml. Sealing the 2 ends of the tubings did not change the release rate significantly. Coventry and Newman (1982), used tubes filled with 20% chlorhexidine in the periodontal pockets of 11 patients, and reported a marked reduction in bleeding on probing and crevicular fluid flow after 7 days. Later, Khoo and Newman (1983) used dialysis tubing containing 0.5% metronidazole in a parallel design study. Three groups of patients received either metronidazole tubings or acrylic strips containing 40% metronidazole or subgingival irrigation using 0.2% chlorhexidine as adjuncts to root planing. Tubings and strips were replaced every 7 days for a total period of 28 days. Irrigation was performed by the patients once daily for the same period. Control sites from the same patients received only scaling and root planing. Plaque samples showed that metronidazole tubings and the metronidazole strips both reduced the proportion of spirochaetes. The effect of chlorhexidine irrigation was significantly less

than the other 2 treatments. Root planing alone had the least effect. The clinical effect of treatment by metronidazole tubes or chlorhexidine subgingival irrigation were further compared by wan Yusof *et al.* (1984), where 4 weekly applications of tubes or once daily irrigations for 28 days were performed for each patient as adjuncts to root planing. At day 84, the pocket depth reductions were 1.6 and 1.1 mm for tubing and irrigation groups respectively. The results of this study should be interpreted cautiously because no control groups were used in the study.

1.8.5.2 Acrylic strips

Addy *et al.* (1982) described the development of polymethyl-methacrylate strips containing various concentrations of chlorhexidine acetate or 40% tetracycline or metronidazole. The *in vitro* release of the above mentioned substances from the strips were assessed *in vitro* spectrophotometrically. It was reported that both tetracycline and metronidazole strips as well as 30% to 50% chlorhexidine strips continued to release the substance for up to 14 days. However, after initially releasing a high concentration on day 1, a marked fall in release was observed on day 2 with a progressive fall thereafter. Addy, Alam and Rawle (1984) used acrylic strips containing 40% tetracycline in deep periodontal pockets for 2 to 3 days. The proportions of motile organisms and Gram negative rods were reduced. An initial

reduction in pocket depth was reported in sites treated using metronidazole or tetracycline strips but after 3 months a return toward baseline was observed in the tetracycline group (Addy, Langeroudi and Hassan, 1985). Addy *et al.* (1988) in a 3 month parallel design clinical study compared the efficacy of acrylic strips filled with either metronidazole, tetracycline or chlorhexidine as the sole therapy with scaling and root planing alone. After 14 weeks the metronidazole strips and scaling alone had been more effective in improving the clinical parameters than the other 2 treatments. Wade *et al.* (1992) reported that the placement of acrylic strips impregnated with tetracycline resulted in an increase in the proportion of tetracycline resistant bacteria in the treated sites. In other groups using chlorhexidine or metronidazole strips or root planing, no such effect was noticed.

1.8.5.3 Ethyl cellulose films

Ethyl cellulose is another substance which has been extensively investigated for its potential to act as a sustained release system in periodontal pockets. In a study by Friedman and Golomb (1982), chloroform or ethanol was used to dissolve the ethyl cellulose polymer and allow the chlorhexidine to become incorporated into its structure. In addition, polyethylene glycol was used to modify the release rate. Within 10 days, 95% of the chlorhexidine was released *in vitro*. Soskolne *et al.* (1983) reported that 60% of the drug was released within 6

days *in vivo*. However, bacteriological sampling indicated that, following a transient reduction, the level of spirochaetes returned to baseline values after 2 weeks. Golomb *et al.* (1984) incorporated 30% metronidazole in the ethyl cellulose films. More than 70% of the drug content was released into water *in vitro* during the first 24 hours. The *in vivo* kinetics was found to be similar to the *in vitro* release into water. Stabholz *et al.* (1986) used the ethyl cellulose films containing chlorhexidine for 3 consecutive applications, each for 3 days. Until the end of the 11 week period of the study, pocket depth and bleeding on probing, as well as microbiological parameters were reported to be markedly reduced. Stabholz *et al.* (1991) used this system for patients who still had pockets > 5 mm, 2 months after root planing. They used a split mouth design whereby the contralateral quadrants received routine maintenance therapy. The test treatment included 3 applications of ethyl cellulose films containing chlorhexidine which were replaced every 3 days without any adjunctive root planing. Both treatments were repeated every 3 months for a total period of 2 years. The best results in both groups were observed at the 6 and the 9 month visits with an average pocket depth reduction of 2.3 mm in the test group versus 1 mm reduction in the control group. Surprisingly, the amount of attachment gain was also 2.3 mm indicating that there was pure attachment gain and no recession. This system, whilst

promising, involves 3 visits every 3 months, a regime which normal patients find difficult to comply with.

1.8.5.4 Collagen films

Minabe *et al.* (1989a) described the development of a local drug delivery system consisting of collagen films and tetracycline. Atelocollagen was dissolved in water and basic tetracycline was added plus HCl to adjust the acidity. Acetone plus glutharaldehyde saturated with tetracycline was added to the first preparation to induce cross linkage in the molecular structure. The dried product was cut into strips. Analysis of GCF showed that until day 10, tetracycline concentration was above the MIC of tetracycline for the anaerobic bacteria. The authors reported that the prolonged release action was due to tetracycline being incorporated into the cross linkage of the polymer. However, a treatment regime consisting of four weekly applications of the tetracycline collagen films produced only transient improvements in clinical and microbiological outcomes (Minabe *et al.* 1989b; Minabe *et al.*, 1989c). A relatively short lived response to the treatment in the above mentioned studies is not surprising, and most probably is due to the absence of scaling in the study design and the calculus being left intact. Realising this concept, Minabe *et al.* (1991) performed a clinical study of treatment of periodontal furcation lesions. A split mouth design was implemented with the treatment groups including: scaling and root

planing alone, insertion of tetracycline collagen films alone, once a week for 4 weeks, root planing plus collagen films and untreated controls. After 8 weeks, in the scaling alone group, a15% reduction in bleeding on probing took place together with a 2 mm reduction in pocket depth and a 1.1 mm attachment gain. The result of combination therapy was reported to be an 80% reduction in bleeding on probing, a 2.7 mm reduction in pocket depth and a 2 mm attachment gain. Although the results of this study indicate a positive outcome in the short term, the evidence of long lasting results is lacking. Moreover, the treatment had the disadvantage of several applications being necessary.

1.8.5.5 Monolithic fibres

In 1983 Goodson *et al.* evaluated the physical behaviour of 6 polymers as vehicles to carry tetracycline and slowly release it into gingival fluid. The materials included polyethylene, polypropylene, polycaprolactone, polyurethane, cellulose acetate propionate and ethylene vinyl acetate. The polymers were melted, mixed with 25% tetracycline, spun, extruded and cooled to form cylindrical and monolithic fibres with a diameter of 0.5 mm and containing 322 µg tetracycline/cm. *In vitro* and *in vivo* studies showed that all the polymers except for ethylene vinyl acetate (EVA) released their tetracycline content either too fast or too slowly. Ethylene vinyl acetate maintained its release until 9 days with an

initial concentration of about 600 µg/ml and a half life of 13 hours. Later, Goodson *et al.* (1985a) reported a sustained concentration of 600 µg/ml throughout the 10 day period of the study. This report, in contrast to the previous findings of Goodson *et al.* (1983), indicated that the half life was infinite, at least during the 10 day period of the study i.e. a zero-order delivery occurred. The zero-order kinetics of the monolithic tetracycline fibres was later confirmed by Tonetti, Cugini and Goodson (1990), but a sustained GCF concentration of 1590 µg/ml was reported this time, which was more than 2.5 times the level of the previous report. Using scanning electron microscopy (SEM) they described the presence of the tetracycline crystallites on the surface of the carrier matrix. Four hours after exposure of the fibres to the water, the superficial crystallites were no longer present leaving behind narrow channels and niches. The physical principles of the rate of release of solid drug dispersed in solid matrices has been described several years ago (Higuchi, 1963). Once the fibre is placed in the subgingival area, the superficial crystallites dissolve quickly by simple diffusion. Thereafter, the dissolution takes place through narrow channels created and progressively elongated by the dissolution of more superficial crystallites. This progressive elongation causes the solubilisation front i.e. the surface area of the drugs under process to remain constantly low. Obviously the establishment of such a tortuous structure

depends on the connectivity of the drug crystallites, which in turn results from the melted matrix being impermeable to the drug. After fibre removal, the concentration of the drug decreases exponentially with a half life of 4.5 hours (Tonetti *et al.*, 1990). The drug concentration remained above 50 µg/ml for a total period of 11 days. The corresponding time period for 1% and 10% tetracycline solutions were 21 hours and 66 hours respectively when used to irrigate the pockets (Tonetti *et al.*, 1990).

Goodson *et al.* (1985b) reported a treatment consisting of root planing plus tetracycline fibres for adult periodontitis patients, to be superior to root planing alone, and there was approximately 1 mm attachment gain after 6 months. It has been reported that the tetracycline released from the monolithic fibres could penetrate from 1-20µm into the soft tissue wall (Ciancio, Cobb and Leung, 1992) and up to 10 µm into the cementum and dentinal tubules (Morrison *et al.*, 1992). The presence of bacteria in the dentinal tubules of the radicular dentine and the soft tissues of the pocket has been reported in the literature. These micro-organisms might be responsible for disease recurrence after mechanical therapy. However it is not clear whether a slight penetration of tetracycline into the soft and hard tissues could inhibit such invading bacteria. In fact the study of Mandel *et al.* (1986) suggested that tetracycline fibres failed to

inhibit *A.a.*, a micro-organism reportedly capable of invading the soft tissues in LJP lesions. They reported that monolithic tetracycline fibres when used as the sole treatment of the juvenile periodontitis lesions, failed to prevent further attachment loss and eradicate *A.a.* The percentage of *A.a.* positive sites was increased. These sites were then successfully treated using surgery plus systemic doxycycline therapy for 14 days. Goodson *et al.* (1985b) reported that scaling alone produced less reduction in the motile and spirochaete counts as compared to treatment with monolithic tetracycline fibres. The clinical and microbiological effects of tetracycline fibres were further investigated by Goodson *et al.* (1991a,b,c) in a multi-centre trial. Using a split mouth design, 107 patients in 5 centres were treated and monitored for a period of 60 days. One site in each quadrant was selected and treated. Treatments consisted of scaling and root planing alone, placement of monolithic tetracycline fibres for 10 days and placement of placebo fibres. One quadrant remained untreated. Ten days prior to the baseline, all the sites were treated by supra-gingival scaling. Measurements included microbiological sampling and recording of pocket depth, attachment levels and bleeding on probing. Tetracycline fibre therapy was reported to be significantly superior to the other treatments. A rather large change in the 2 placebo and untreated groups was observed and attributed to all the sites being supra-gingivally instrumented before the

baseline. Microbiological monitoring of sites using DNA probes at days 0 and 10 showed that both the scaling and tetracycline fibre groups equally, and significantly, reduced the proportion of periodontal pathogens (Goodson *et al.*, 1991c; Maiden *et al.*, 1991). Goodson and Tanner (1992) reported that the observed increase in the levels of tetracycline resistant bacteria was not due to development of plasmid mediated multiple antibiotic resistance, but simply due to an increase in the proportion of gram positive cocci.

Rapley *et al.* (1992) reported that after the tetracycline fibre treatment of at least 8 teeth per patient, the serum levels of tetracycline did not exceed 0.1 μg/ml. They reported no serious adverse effects associated with the treatment. The use of tetracycline fibres as an adjunct to root planing was investigated by Heijl *et al.* (1991). Using a 4 quadrant split mouth design the combination of root planing with tetracycline fibre therapy was reported to be superior to either of the sole treatments, although not significantly. Bacterial sampling of the sites confirmed the clinical findings. Newman *et al.* (1994) conducted a multi-centre trial on the efficacy of tetracycline fibres as adjunct to root planing on a maintenance population. After 6 months, the improvement in the clinical parameters of patients who received scaling plus tetracycline fibres was significantly higher than those treated by scaling alone.

1.8.5.6 Supragingivally applied anti-plaque agents

Chlorhexidine, quaternary ammonium compounds such as cetyl pyridinum chloride, phenolic compounds such as triclosan, flourides, oxygenating agents, metallic ions such as zinc, copper and tin, and enzymes such as amyloglucosidase and glucosoxidase have been used in oral rinse and tooth paste formulations. Since the efficacy of chlorhexidine in plaque control was first demonstrated by Löe and Schiött (1970), it is still perhaps the most effective anti-plaque agent for oral use. Chlorhexidine is a dicationic molecule which can bind onto the oral surfaces, while still retaining its bacteriostatic activity. This property permits chlorhexidine to retain bacteriostatic levels for prolonged periods following application (Rölla and Melson, 1975). Oral rinse or supragingival irrigation of the gingival margin are not effective ways of delivering the irrigants into the pocket (Pitcher, Newman and Strahan, 1980; Braun and Ciancio, 1992).

1.8.5.7 Subgingival irrigation

Many studies have been conducted to test the efficacy of irrigation of periodontal pockets using antibacterial solutions. It is beyond the scope of this thesis to review in detail the effect of irrigation on periodontal disease. In general, the effect of irrigation as an adjunct to conventional therapy has produced transient or unremarkable results (Jolkovsky *et al.*, 1990; Lander *et al.*, 1986; Wennström *et al.*, 1987; Rosling *et al.*, 1986).

The results of studies on the penetration of the irrigant by subgingival irrigation devices are not consistent. However, most studies demonstrated that with irrigation therapy the antibacterial agent fails to reach the plaque front completely (Pitcher, Newman and Strahan, 1980; Eakle, Ford and Boyd, 1986; Silverstein *et al.*, 1988; Braun and Ciancio, 1992; Boyd, Hollander and Eakle, 1992). Antibacterial solutions with high substantivity such as tetracycline, in spite of lack of complete penetration, could be absorbed into the cementum surface and released over time, and so eventually reach the plaque front through diffusion into the deeper area of the pocket (Stabholz *et al.* 1993a). Positive results reported by some authors who tested this approach supports this theory (Christersson, Norderyd and Puchalsky 1993). Chlorhexidine, while effective against most subgingival organisms *in vitro*, has less efficacy *in vivo*, presumably due to the absorption by serum proteins in the GCF (Hjelford, Rölla and Bonesvoll, 1973), and poor absorption of chlorhexidine in deeper layers of plaque.

1.8.5.8 Biodegradable systems and gels (metronidazole gel, minocycline gel and other resorbable systems)

The main advantage of locally applied gels used as a controlled delivery mechanism is their relatively easy application and the lack of any need for professional removal of the vehicle. Nevertheless, the use of

antibacterial agents in the form of gels as local delivery systems will not necessarily increase the substantivity of the drug. Unless the drug itself is a substantive material in the subgingival environment or the gel vehicle actively contributes to the retention of the drug, the drug will simply be washed out. A gel with sufficient flow to be syringeable into the pocket is quickly cleared from the area. Oosterwaal, Mikx and Reaggli (1990) reported that when a fluorescein gel was injected into the pocket, 50% of it was cleared during the first 12 minutes.

Metronidazole gel: In 1992 Norling *et al.* introduced a drug formulation for use in periodontal pockets which consisted of 25% metronidazole carried in a vehicle gel. The drug is a mixture of sesame oil, glycerol mono-oleatè (GMO) and metronidazole benzoate. The vehicle materials are polar lipids, which are insoluble, but swell in water. Contact with water results in the formation of a reverse hexagonal form. In this form, the channels for the passage of water are closed, resulting in the retardation of the solubility of the metronidazole benzoate. When the mixture comes into contact with water, the metronidazole benzoate on the surface dissolves immediately. As the hydration progresses, the remaining metronidazole-free vehicle (sol) on the outer surface gradually turns into the reverse hexagonal state (gel) which controls metronidazole release from within. Stolze and Stellfeld (1992) reported that the systemic absorption of

metronidazole after application of the gel in an average of 10 periodontal pockets per patient, was lower than that of a single dose of one 250 mg metronidazole tablet. The excess amount of gel which is normally swallowed was reported to be 60% of the total amount applied. After the application of the gel, there is a rapid decrease in GCF concentration of the drug after 4 hours (Stoltze, 1992). The relatively rapid fall in GCF levels of metronidazole after gel application indicates that the system lacks the substantivity, even if its concentration in the GCF passes the MIC thresholds at the very early stages of treatment as reported by Stoltze (1992). Moreover, it was reported that after 24 hours, 50% of the sites still showed metronidazole levels greater than 1 μg/ml (Stoltze 1992). However, care should be taken when interpreting this data. Five cartridges were used for each patient, i.e. 1.25 g metronidazole per patient. After systemic administration of 250 mg of metronidazole a peak plasma concentration of 5 μg/ml is observed. This is usually seen 4 hours after administration. Therefore with a plasma half life of 8 hours (Neidle, Kroeger and Yagiela, 1985), the serum concentration, which is equal to the GCF levels of the metronidazole, 24 hours after application of 5 cartridges is expected to exceed 3 μg/ml. Therefore even if no controlled release action exists and only systemic absorption via direct absorption and swallowing occurs, the observation after 24 hours of 50% of sites showing levels higher than 1 μg/ml is quite expected. These data

indicate that sustained release action in the metronidazole gel system is minor if there is any at all. After the proof of efficacy of metronidazole as a local drug using a ligature-induced periodontitis model in beagle dogs, (Klinge *et al.*, 1992a), Klinge *et al.* (1992b) evaluated the metronidazole delivery system using varying concentrations of metronidazole and varying treatment regimes. They reported that the best outcome is achieved if it is used at 25% concentration, once a week for 2 weeks. Metronidazole gel formulation is now commercially available as disposable 'intraligamentous type' injection syringes containing 1 g of gel which is injected into the pocket using a blunt needle (Elyzol, Dumex, Copenhagen, Denmark). Pedrazzoli, Kilian and Karring (1992) compared the clinical and microbiological effects of 25% metronidazole gel alone with root planing alone, using a split mouth design. Both treatments i.e. gel therapy and root planing were reported to be effective in improving the baseline parameters. The gel therapy was found a little better, although not significantly, than the mechanical therapy. However, the data on bleeding on probing indicated that a trend of a return towards the baseline appeared in both groups at week 12 until the end of the 24 week period of the study. Surprisingly, only 33% of the pockets showed bleeding on probing at baseline. The absence of bleeding on probing is one of the most reliable indicators of periodontal health (Lang *et al.*, 1986; Lang *et al.*, 1990). This indicates that the majority

of sites in the quoted study were probably not periodontally diseased. The inclusion of non diseased sites in a study of therapeutic modalities for periodontal disease will mask any difference between the groups and dilute the treatment effect. A similar clinical design but on a larger scale was employed by Ainamo *et al.* (1992) who evaluated 206 patients in a 6 month study in 9 centres. In contrast to the study of Pedrazzoli *et al.* (1992), the scaling was found to be significantly more effective than the metronidazole gel. The pocket depth reduction was 1.5 mm and 1.3 mm for scaling and gel therapy respectively. The authors considered the difference clinically unimportant. The patients were excluded if they had had periodontal treatment other than supra-gingival scaling in the past 6 months. It should be mentioned that the application of gel or fibre into the pocket is virtually impossible if supra-gingival calculus is present at the site, thus making supra-gingival scaling inevitable. On the other hand, by inclusion of newly supra-gingivally scaled patients in the study, one may argue that the pocket depths of these patients might continue to improve due to the supragingival scaling, after they have been measured at baseline. Such a phenomenon was reported by Goodson *et al.* (1991b). While such a design would not induce any imbalance in a study which utilises an untreated control group (because any change due to pre-baseline treatment would be reflected in the untreated control too), it could lead to misconclusion

if there is no untreated control group in the study protocol. Another way round this problem would be comparing the effect of supra-gingival scaling plus gel therapy with supra- plus sub-gingival scaling.

Minocycline gel: A series of studies in Japan were conducted to develop an antimicrobial ointment for periodontal application. A formulation of 2% minocycline in lipid was developed as a viscous gel for application by the dentist directly into the crevicular space (Satomi *et al.*, 1987; Naora *et al.*, 1987; Kurimoto *et al.*, 1987; Isoshima *et al.*, 1987; Ishikawa *et al.*, 1988; Ueda *et al.*, 1988; Kurimoto *et al.*, 1988; Murayama *et al.*, 1988; Murayama *et al.*, 1991). Briefly, these studies reported that: a) due to a wide range of activity and high substantivity in the GCF, minocycline was the best candidate for use as a local antibacterial agent for periodontal therapy; b) the systemic concentration following the local application of the ointment was minimal; c) a single dose of minocycline ointment only had a transient effect on the bacterial population which returned to baseline values after 2 weeks; d) to obtain a clinical improvement, the ointment had to be applied three to four times at one or two weekly intervals. There was no difference between the outcomes of three fortnightly and four weekly applications.

The persistence of sublethal levels of wide spectrum antibiotics such as minocycline in localized sites of the body for long periods may predispose those sites to the emergence of resistant bacteria (O'Connor, *et al.*, 1990). However, no difference was observed between systemic and locally applied minocycline on their effect of increasing the bacterial resistance to minocycline (Preus *et al.*, 1995). In 1993 van Steenberghe *et al.* conducted a clinical and microbiological study using the 2% minocycline ointment as an adjunct to scaling and root planing. A total of 103 patients with at least one periodontal pocket were included in a parallel design study. One half of the patients received scaling and root planing plus minocycline ointment, the other half scaling and root planing plus a placebo. The gel application was repeated at weeks 2, 4 and 6. Measurements were taken at baseline and after 2, 4, 6 and 12 weeks. Both clinical and microbiological measurements were taken. By week 12, mean reductions in probing depth of 1.7 mm and 1.4 mm were observed, in test and control groups respectively. The attachment gain was 0.8 mm for both groups. The bleeding indices were 1.2 and 1 respectively. The minocycline gel group showed 13% fewer pockets with detectable *P.gingivalis*, *P.intermedia* and *A.actinomycetemcomitans*. The reduction in pocket depth and the microbiological improvements were significantly different between the 2 groups. Minocycline ointment is now commercially available (Dentomycin, Cyanamid, Lederle, U.K.). The

manufacturer's recommendation is 3 or 4 bi-weekly applications of the gel after a single scaling and root planing. 500 mg ointment containing 2% minocycline is loaded in a disposable polypropylene syringe with a plastic contra-angle tip. The material is unstable at room temperature and should be kept in the refrigerator. Preus *et al.* (1993) reported that the transmission of minocycline resistant bacteria between pockets within the same mouth was likely, therefore they recommended that after application of the ointment into each pocket, the syringe tip should be cleaned with ethanol.

Other drug delivery systems with resorbable vehicles: Noguchi, Fukuda and Ishikawa (1988) described the development of a resorbable delivery system. Tetracycline or chlorhexidine were dissolved in ethanol and hydroxypropylcellulose powder was added to the solution. The end product was formed into strips which could be inserted into periodontal pockets. A treatment regime of 3 applications for one week was advocated. This approach was tested in 2 separate split mouth design studies using tetracycline as well as chlorhexidine strips. At week 3, the pocket depth was only reduced in the sites treated with tetracycline strips. However, the pocket reduction was transient and a trend towards baseline was observed 2 weeks after treatment.

Another antibiotic, ofloxacin, was also used in a similar manner by Higashi *et al.* (1990). Polymethacrylic acid particles containing ofloxacin were embedded in a matrix of hydroxypropylcellulose which also contained ofloxacin. This resulted in a biodegradable system (PT-01) which carried 10% ofloxacin. The authors reported ofloxacin concentrations above 2 μg/ml in the GCF until 7 days after insertion of the system. Kimura *et al.* (1991) tested the material on 27 subjects using a split mouth design. The treatment was performed weekly for 5 weeks. Microbiological samples were taken at weeks 0, 2, 3 and 6. In addition to the drug therapy, all test and control sites received supragingival scaling during the first 2 weeks and subgingival root planing thereafter. The microbiology results showed that during the first period i.e. supragingival scaling plus drug therapy, a significant reduction of motile rods, spirochaetes and black pigmented *Bacteroides* took place in the test sites only, whereas after 2 weeks i.e. following subgingival scaling plus drug therapy, no significant difference between test and control sites was evident. Yamagami *et al.* (1992) used the ofloxacin delivery system in periodontal pockets once a week for 4 weeks. No adjunctive mechanical therapy was used in either the test and control sites. It was reported that after 4 weeks, probing depth reductions of 1.5 mm vs. 0.6 mm occurred in test and control sites respectively. The reductions in bleeding on probing were 62% and 10% correspondingly. The

usefulness of local treatment of periodontal lesions by ofloxacin had previously been reported by Miyake *et al.* (1988) and Kametaka, *et al.* (1989).

Okuda *et al.* (1992) described a biodegradable delivery system to release minocycline into periodontal pocket. A powder formulation consisting of minocycline HCl microencapsulated in a biodegradable polymer, poly(glycolide-co-dl-lactide) was placed into the pockets using a plastic syringe. Subgingival scaling combined with a single dose of the drug was compared to scaling alone in a parallel design study. It was reported that after 6 months, the microbial flora was more favourable for periodontal health in the combined scaling and minocycline therapy group as compared to the scaling alone group. However, Jones *et al.* (1994) after a 6 month follow-up reported that the system, when used as an adjunct to root planing, failed to improve the pocket depth significantly over scaling alone. The attachment gain in the scaling group was reportedly higher than in the group treated with the adjunctive drug.

Eckles *et al.* (1990) used a 40% mixture of tetracycline in petrolatum in a split mouth design study. One quadrant received the tetracycline mixture. The other 3 quadrants received root planing alone, petrolatum without tetracycline and no treatment. At week 12, only the pocket depths of sites treated by root planing alone

showed a significant reduction compared with baseline. Bleeding on probing and dark field microscopy data showed a transient reduction at week 4 in root planing and tetracycline groups, but at week 12 these parameters were almost similar between all groups. Unsal, Akkaya and Walsh (1994) reported that 40% tetracycline in petrolatum as an adjunct to root planing provided significant benefit over scaling alone. When the same system was used as an adjunct to root planing for the treatment of LJP patients, no benefit over scaling alone or scaling plus chlorhexidine gel was achieved (Unsal, Walsh and Akkaya, 1995).

A system consisting of 5 mm x 5 mm square pieces of type I collagen impregnated with 5% metronidazole as an adjunct to root planing was reported to produce significantly greater improvement in clinical indices compared to root planing alone for a period of up to 3 months after treatment (Hitzig *et al.*, 1994).

The use of biodegradable materials in local delivery systems could facilitate the treatment of periodontal lesions by omitting the need for removal of the system after the therapeutic period. However, since usually more than one visit for the drug application was required, there was no advantage over the non-resorbable materials which needed a second visit for their removal.

1.9 A critical conclusion of the literature review

The existing reports in the literature concerning the efficacy of using antibiotics in the treatment of periodontal disease is controversial. The routine use of systemic antibiotics for the treatment of adult periodontitis is not justified. Root debridement with or without surgical access are usually successful in the treatment of adult periodontitis. Some data support the use of certain antibiotics for LJP or refractory periodontitis. However, reviewing the reports indicative of a successful response to mechanical therapy alone in these groups of patients (Waerhaug, 1977; Christersson *et al.*, 1985; Saxén *et al.*, 1986), it seems reasonable to firstly take these patients through mechanical therapy alone. A diagnosis of refractory periodontitis should not be made unless all the possible causes of failure are ruled out. The quality of the patient's oral care as well as the professional skills of the operator are principal prerequisites for treatment success. Similarly in considering the treatment of recurrent periodontal disease the failure should be weighed against the quality of the maintenance programme. If the consequence of an unsuccessful response is considered so serious that it justifies the risk of potential side effects of systemic antibiotic therapy, then antibiotic therapy could be advocated after confirmation of antibiotic susceptibility.

The aetiology and pathogenesis of periodontal disease is complex. If there are true specific pathogenic bacteria among the inhabitants of the subgingival area, then it may be assumed that the occasional lack of response to mechanical therapy is due, in part, to the pathogenic bacteria re-populating the root surface from their permanent reservoirs in the oral cavity such as the tonsils, the dorsum of the tongue, the oral mucosa, furcation lesions and dentinal tubules (Adriaens, De Boever, and Loesche, 1988a; Adriaens *et al.*, 1988b). In such cases theoretically, the prescription of an antibiotic may be useful in eliminating the pathogen and therefore arresting the progression of the disease.

Two questions arise here. Firstly, the concept of a reservoir in the soft tissues is still controversial. If it does exist, then naturally adjunctive systemic antibiotics are a more effective treatment option than root planing or local drug delivery systems. However, if the dentinal tubules are a source of re-infection of the pockets, they appear to be inaccessible to both local and systemic antibiotic therapy as well as root planing. In this case, surgical pocket elimination seems the only logical way of preventing the recurrence of the disease. The second question is whether periodontal pathogens are indigenous or exogenous. A large amount of evidence indicates that some bacteria are strongly associated with disease progression while their elimination correlates

with inhibition of the disease (Newman and Socransky, 1977; Slots, 1976; Slots, 1977; Spiegel *et al.*, 1979; Tanner *et al.*, 1979; White and Mayrand, 1981; Zambon, Reynold and Slots, 1981). However, there is also much evidence indicating that these suspected pathogens can also be found at healthy sites. If the suspected pathogens are in fact members of the normal flora, they may re-populate their natural habitat shortly after the cessation of antibiotic therapy. If this occurs, the disease may recur as reported by Kornman and Karl (1982).

In an attempt to provide answers to the question of bacterial specificity in the pathogenesis of periodontal disease, Socransky and Haffajee (1992 and 1993) stated that several conditions should be met for the initiation of a periodontal lesion by pathogenic bacteria. In addition to the presence of the pathogenic micro-organism, the quantity of pathogens should reach a threshold level; beneficial micro-organisms should also be absent or few in number; the pathogen(s) should be of an appropriate virulent clonal type; the host immune response should be impaired and finally the environmental conditions such as iron concentration, temperature, etc. should be optimal. In this context, one could conclude that since the initiation of the disease is the result of activity of specific bacteria, the elimination of these bacteria using antimicrobial agents might be justified. On the other hand, one may argue that if the prerequisite for the

initiation of the disease is so complex and dependant on several interactive factors, then even slight disruption of the ecosystem could stop the pathogenic process. Non-specific debridement should be enough to stop the disease, so long as only one of the above listed conditions are altered in favour of a healthy situation. The complexity of the aetiopathogenesis of periodontal disease still exists and the present literature to date does not provide an answer as to whether or not systemic antibiotics should be used in the treatment of recurrent and refractory periodontitis.

The results of investigations on different local drug delivery systems indicate that:

a) The use of these systems as the sole treatment of periodontal disease has often been found to be inferior to scaling alone. In those studies which reported the superiority of the system as the only treatment to scaling and root planing alone, the benefit has been either transient with a subsequent recurrence of the disease, or lacking the evidence of long lasting improvement.

b) The use of these systems as adjuncts to root planing in untreated periodontal pockets as a routine treatment modality has often brought little or no benefit over scaling alone. In the studies in which statistically

significant advantage over scaling alone were reported, the difference has been clinically unimportant.

c) The pharmacokinetic data indicate that very few systems are capable of maintaining a high drug concentration in the pocket over a sufficient length of time. However, in none of the systems is the drug able to penetrate deeply into the soft tissue adjacent to the pocket.

d) It seems that in most local delivery systems, the systemic uptake of the drug is minimal. Therefore, most adverse reactions observed following systemic drug therapy, including the chance of development of bacterial resistance should remain limited to the periodontium when local drug delivery systems are used.

e) If the non-resorbable systems are used, at least 2 visits are required; one for insertion and another for removal of the system. On the other hand, most resorbable system should be applied at least twice. Together with the price of the systems, these considerations should be taken into account when evaluating the cost effectiveness of delivery systems for the treatment of patients.

f) Some recently published data indicate that some systems may have a potential synergistic effect with scaling and root planing, when used in pockets which showed signs of recurrence of disease in spite of proper treatment and

adequate home care and maintenance therapy (Stabholz *et al.*, 1991; Newman *et al.*, 1994). However, at present the evidence of efficacy of other systems in this population of patients is lacking.

g) Due to large variability in the clinical methods of measuring the treatment outcome, a comparative evaluation of these systems using the existing literature is difficult and inconclusive.

Since the first local drug delivery system was developed, many clinical trials have been conducted to test the value of these systems. A number of these systems are now commercially available for use in clinical practice. However, to date, no direct controlled comparison of these systems has been made. At present, the need for such research is more evident than at any other time given the widespread marketing and usage of these systems in specialist and general practice.

1.10 Laser

1.10.1 General introduction to lasers

Laser energy has been purported as a further alternative or adjunct to scaling and root planing (Midda, 1992; Myres, 1991). In theory, laser energy is capable of ablating and vaporising microbial plaque and calculus. During past few years a number of laser systems have been marketed for several dental applications including periodontal applications.

1.10.2 Production of laser beam

If a molecule of a material is bombarded by photons of the correct size, the molecule will attain a higher energy level and the photons are absorbed. If molecules, the majority of which are in a higher energy level, are bombarded by certain photons some new photons are released. These new photons and the original ones will strike some other excited molecules and again some new photons with the same size and path are emitted and a cascade effect occurs. The photons can be accelerated by means of a photon resonator which consists of a pair of parallel mirrors. If one of the mirrors is semi-reflecting, the accelerated photons can ultimately exit from the container and a parallel sided collimated beam results. Because all the photons are of the same size and energy, the beam will be only of one frequency (i.e. monochromatic) and since all the photons are in the same phase, the beam is coherent and intense. This effect is

called 'light amplification by stimulated emission of radiation' (LASER).

The primary differentiating characteristic between various laser machines is the laser medium which is the original source of the excited molecules. The medium may be either a solid crystal as used in Nd:YAG lasers or a gas such as CO_2. The wavelengths of the lasers are different, depending on the media used to produce the beam. The first laser was made using a bar of synthetic ruby (Maiman, 1960).

1.10.3 Laser interaction with tissues

The interaction of laser radiation with the target depends on a) various properties of the target e.g. chemical structure and density and specific absorption; and b) the properties of the laser radiation e.g. wavelength, energy density and pulse duration. This interaction could be either thermal or non-thermal. *Thermal effect* occurs when the absorbed energy causes an increase in the internal lattice vibration, leading to heat generation. *Photodisruption* occurs if very high energy and short pulsed radiation is emitted to the tissue. The material is ionised by the strong electric field of the beam, resulting in a hot, electrically charged gas, known as plasma. Since, the plasma absorbs all the energy from the incident beam, the generated heat would be minimal. The temperature in the plasma fluctuates as the result of

laser beam, resulting in vibration of electrons within plasma which leads to generation of audible shock waves. This so-called photoacoustic waves are responsible for the mechanical breaking apart or shattering of the target material (Miserendino, Levy and Miserendino, 1995).

1.10.4 Types of lasers

There are basically two types of lasers in the medical field which are referred to as hard and soft lasers. Hard lasers emit a concentrated and high powered radiation for use on the tissues. The energy of the photons is much higher than that of soft lasers. There are many types of hard laser such as Ar-F-excimer, Xe-Cl-excimer, Kr-F-excimer, Xe-F-excimer, Nd:YAG, Ho:YAG, Er:YAG, and CO_2 lasers of which the two most commonly used in dentistry are the CO_2 and Nd:YAG lasers. Soft lasers emit a low-power beam with the wavelength being in the visible or near infra-red region of the spectrum. Any biological effect occurring after tissue exposure to these types of laser are due to the direct effect of radiation rather than as a result of heating (*photodynamic interaction*).

1.10.2 Nd:YAG Laser

The Nd:YAG laser emits a wavelength of 1064 nm which is transmissible through an optic fibre, making it easier to handle in the oral cavity. The laser medium in this type of laser includes a solid crystal of yttrium aluminium garnet dotted with neodymium. Since 1064 nm is not in the

visible spectrum, it also needs an accessory He-Ne laser as an aiming beam. An Nd:YAG laser beam can penetrate water and soft tissues which contain a high percentage of water up to a considerable depth, before its energy becomes attenuated (Harris and Pick, 1995). This property of the Nd:YAG laser makes it more suitable for surgical removal of abnormally vascularised lesions which tend to bleed during ordinary surgery with a scalpel (Halldorsson & Langerholc, 1978).

1.10.3 The effects of lasers on the pulp

The effect of laser energy on the dental pulp has been evaluated (Adrian, Bernier and Spraque, 1971; Melcer, Chaumette and Melcer 1987; Serebro *et al.*, 1987; Friedman, *et al.* 1991; Leighty *et al.*, 1991; Frentzen, Koort and Thiensiri, 1992; Miserendino *et al.*, 1993; White, Fagan and Goodis, 1994). Due to the diversity of the laser techniques, energy durations and wavelengths, the results are often incomparable. However, it appears that the energy of the Nd:YAG laser, due to the absorptive characteristics of dental hard tissues, could penetrate deeply through enamel and dentine and reach the pulp chamber (Launay *et al.*, 1987). Bahchall, *et al.* (1993) reported focal haemorrhage and disruption of odontoblastic layer in the dental pulp of dogs when the enamel was treated by Nd:YAG laser energy. The pulpal effect of the Nd:YAG laser, was studied by White *et al.* (1994). The results indicated that if the thickness of dentine between

the laser impact and the pulpal tissues was 2 mm, energy settings as low as 70 milli-Joules (mJ), at 10 pulses per second (pps) for 30 sec could produce a temperature rise of 5.8°C in the pulp. If the thickness was 1 mm the temperature rise was about 10°C and 17°C after energy settings of 70mJ and 100mJ at 10pps for 30sec respectively. It is important to consider that a temperature rise of 5.6 °C, causes 15% of the teeth to become non-vital as reported by Zach and Cohen (1965). The distance between the cementum surface and the root canal is often close to 2 mm and it may even be less, especially in inter-proximal root concavities and in periodontally diseased teeth which have been subjected to long-term tooth structure loss due to root planing at maintenance visits.

1.10.4 Lasers in periodontics

1.10.4.1 Soft tissue surgery

Lasers have been used for various soft tissue surgery techniques in periodontics such as gingivectomy (Pick, Pecaro and Silberman 1985; Hylton, 1986; Barak and Kaplan, 1988), flap incision (Pecaro and Garchume, 1983), sulcus deepening (Pogrel, 1989), frenectomy (Pogrel, 1989) and gingival curettage (Gold and Vilardi, 1994). Available evidence shows that bleeding during laser surgery is less than that when using a scalpel to perform surgery (Pogrel, 1989; Barak and Kaplan, 1988; Pecaro and Gachine, 1983; Pick *et al.*, 1985; Frame, 1985a; Frame, 1985b, Horch,

Gerlack and Schaefer, 1986; Hylton, 1986; Abt *et al.*, 1987; Frame *et al.*, 1988). This may offer an advantage over traditional techniques when performing surgery on patients with clotting defects or vascular disease (Pick and Pecaro, 1987).

There are a number of reports on the healing of laser treated wounds compared to scalpel wounds (Hall, Hill and Beach, 1971; Fisher *et al.*, 1983; Fisher and Frame, 1984; Abergel *et al.*, 1984; Goultschin *et al.*, 1988; White, Goodis and Rose, 1991). Due to the diversity of these studies in terms of the type of laser, the duration, and the energy settings, the comparison and interpretation of the results is difficult and inconclusive. The reported results are inconsistent. While most authors reported that the scalpel incisions healed more quickly than the laser incisions, a few others found little difference between the 2 methods. It seems that in interpreting the biological effects of lasers on the tissues, each laser treatment with its own parameters such as specific wavelength, energy, pulsation and duration should be specifically and individually considered and assessed. The Nd:YAG laser energy has been reported to delay the ability of skin fibroblasts to synthesise DNA and collagen (Abergel *et al.*, 1984). Most data indicate that re-epithelialization of laser wounds is delayed. Some authors have suggested that this phenomenon might be beneficial in surgical procedures such as sulcus deepening

(Pogrel, 1989), or in preventing the apical migration of the epithelium after periodontal surgery (Midda and Renton-Harper, 1991; Gold and Vilardi, 1994). White *et al.* (1991) reported that the Nd:YAG laser was as effective as a scalpel in periodontal soft tissue surgery techniques. They reported that post-operative pain and inflammation were equal in the scalpel treated and laser treated groups. Gold and Vilardi (1994) reported that the Nd:YAG laser was used successfully to remove the pocket epithelium without inducing necrosis or carbonisation of underlying connective tissue.

1.10.4.2 Treatment of dentinal hypersensitivity

Renton-Harper & Midda (1992) used an Nd:YAG laser for the treatment of hypersensitivity and reported less sensitivity of the exposed roots after application of the laser at energies of up to 50 mJ for 2 minutes. The sensitivity was assessed by patient tolerance of thermal stimuli provided by a cold-air blast. These results were confirmed by Gelsky, White and Pruthi (1993).

1.10.4.3 Lasers and the root surface

The number of well controlled studies evaluating the behaviour of the periodontal tissues to laser radiation is still far from sufficient to provide a proper understanding of this subject. In 1992 the Institute for Laser Dentistry in a clinical manual (unpublished data) claimed that lasers would be capable of enhancing calculus

removal, killing the pocket bacteria and vaporising the necrotic tissues. Research carried out on whether the laser has any place in the treatment of diseased root surfaces, is briefly reviewed below.

The *in vitro* effects of the Nd:YAG laser on subgingival calculus were studied by Tseng *et al.* (1991a) using the scanning electron microscopy (SEM). They used laser energies of 2.0W or 2.75W at 20pps for two or three minutes, and then tested the lased surfaces for ease of calculus removal using a hand curette. They reported heat penetration and damage in localized areas of cementum and even dentine. However, there was a significant difference between the number of curette strokes required to remove lased (3.8 strokes) versus non-lased calculus (6.5 strokes), and it was concluded that although laser treatment *per se* could not remove a significant amount of calculus, it enhanced calculus removal.

In an SEM study, Morlock *et al.* (1992) evaluated the effect of the Nd:YAG laser *in vitro* either alone, or as an adjunct to root planing. They used energies of 1.25W and 1.50W at 20pps for 20sec on a circular area of 3mm diameter. All the treated groups showed changes on the surface of the cementum including: crater formation, surface pitting, charring and tracking. They also reported that the cementum layer of lased surfaces was

peeled off in some areas when subjected to hand instrumentation.

Trylovich *et al.* (1992) studied the biocompatibility of Nd:YAG laser treated root surfaces. They incubated three groups of untreated healthy, lased endotoxin treated, and non-lased endotoxin treated root segments in human gingival fibroblast cultures. The biocompatibility of the surfaces was assessed by the presence of flat fibroblasts (which were considered as firmly attached) and the absence of round fibroblasts (which were believed to be poorly attached) on the root segments. They reported significantly fewer flat fibroblasts in the laser treated group compared to both healthy and non-lased endotoxin treated groups. Although a relatively low energy level (80mJ at 10pps for one minute over a 4x4 mm^2 area) was used, surface alterations of charring, cratering and melting down were observed. Similar results were reported by Tewfik *et al.* (1994) using a modified Nd:YAG laser with a wavelength of 532 nm. Thomas *et al.* (1994) found that if the laser treated cementum surface was root planed after the laser treatment, the number of well-attached fibroblasts increased, but was still below the levels observed in unlased controls, whereas the treatment of the surface using air-powder abrasive after laser treatment resulted in fibroblast attachment almost equal to the unlased controls. They concluded that the adverse effect of laser on the root surface is a superficial phenomenon.

Arcoria *et al.* (1992) used a coaxial CO_2-Nd:YAG laser for irradiation in periodontal surgery on dogs to evaporate granulation tissue from osseous defects and re-contour the osseous irregularities of the alveolar crest. Two different laser treatments were used. The lower energy treatment (energy density = 426.6 J/cm^2) resulted in no improvement when compared to the conventional treatment, whereas the higher energy treatment (energy density = 849.3 J/cm^2) ended up with significantly more loss of attachment and tissue necrosis. This study did not determine which type of laser contributed to the adverse effects on the periodontal tissues.

Ito, Nishikata and Murai (1993), in an SEM study, reported that an Nd:YAG laser was capable of removing the smear layer from the root surface after root planing. The smear layer is a product of root instrumentation and consists of mineral and organic remnants of cementum, dentine and calculus and deposits of bacterial plaque. This layer is thought to act as a barrier between Sharpey's fibres and connective tissue fibres in the cementum. The conventional treatment for the smear layer removal is the application of citric acid. In the above mentioned study, both the laser and citric acid were capable of removing the smear layer. The laser energy densities ranged from 84.9 to 849.2 J/cm^2. The openings of the dentinal tubules were widened after citric acid treatment whereas the laser

treatment did not affect the dentinal tubules. It was stated that since bacterial invasion into the dentinal tubules and inflammation in the pulp tissue are outcomes of widened dentinal tubule openings, the laser treatment might be superior to citric acid treatment. The data obtained from this study would have been more valid if the response of the pulpal tissues had been studied. In addition, the high energy densities used in this study could have affected the biocompatibility of the root tissues.

In a study on the chemical characterisation of lased surfaces, Spencer, Trylovich and Cobb (1992) used Fourier Transform Infrared Spectroscopy. The laser energy settings were 80mJ, at 10pps for 1 minute. After spectroscopy the protein/mineral ratio had decreased when compared to the control sites. They suggested that this might have been due to protein denaturation and production of ammonia as a result of the laser energy. It was pointed out that reattachment might be adversely affected by use of the laser. An attempt was made by Arcoria and Vitasek (1992) to evaluate the efficacy of lasers in calculus removal. Although an Nd:YAG laser was used, the laser pulse duration was far more than that of the American Dental Laser which is available to the dental professions (5 milliseconds versus 150 microseconds). A significant difference in calculus removal between lased and untreated surfaces was reported. However, hand

instrumentation was found to be significantly superior to laser treatment. It was concluded that the absorption characteristics of tissues are most important and that research should be carried out to find a wavelength which is selectively absorbed by the calculus at the cemento-calculus junction. Recently Erbium:YAG laser was evaluated for its efficacy of calculus removal (Aoki *et al.*, 1994). It was reported that the calculus was effectively removed, but a rough and chalky surface was left behind. The SEM confirmed the presence of a very rough surface.

1.10.4.4 The bactericidal effect of lasers

The laser beam is theoretically capable of killing bacteria and providing a germ free field of work. Reid and Stranc (1991) in an animal study reported a significantly more effective role for a CO_2 laser in sterilizing the artificially infected wounds in rabbits when compared with an iodine surgical scrub. However, no data is available to show whether the laser energy sufficient to kill bacteria/reduce the number of bacteria would produce any damage to the host tissues. Further research is necessary to determine the susceptibility of host tissues to the energy levels effective against bacteria. Moreover, longitudinal studies should be designed and carried out to elucidate if a laser treated tissue in the presence of delayed epithelialization will still remain less infected than tissues treated

conventionally. Tseng *et al.* (1991b) in an SEM study, reported that very few periodontal pathogenic micro-organisms remained on contaminated root segments when lased at 1.5W and 10pps for one minute using an Nd:YAG laser *in vitro*. Cobb, McCawley and Killoy (1992) in an *in vivo* study evaluated the antimicrobial effect of the Nd:YAG laser. Laser treatment was performed at 1.75W, 2.25W and 3.00W for one and three minutes either before or after hand instrumentation while some teeth were treated using the laser alone. Pre-treatment and post-treatment samples revealed a marked decrease in the proportion of *A.a.*, *P.g.*, and *P.i.* When two teeth were left *in situ* for seven days, one of them showed an increased number of recovered *P.i.* The authors pointed out that this might be due to recolonisation in laser-induced porosities of the root surface by the bacteria. Recently, Whitters *et al.* (1994) reported that an Nd:YAG laser energy of 80 mJ at 10 pps was capable of killing 90% or more oral bacteria including several suspected periodontal pathogenic species when the laser handpiece was manipulated for 3 minutes in 50ml microbial suspensions each containing single species.

1.10.5 Concluding remarks from the laser literature

Clearly, it is still too soon to consider the laser as a safe and effective tool in the treatment of periodontal diseases. It is not clear whether the laser energy has a consistent effect on the calculus or other hard tissues. If the variation in the absorptive characteristics of

calculus is large, higher energy settings may be necessary to consistently affect calculus. These high settings may not be tolerated by the underlying dental tissues. In addition, the capability of laser to reduce/eliminate pathogenic bacteria from the pocket using moderate and high energies (85 mJ at 20 pps and more) *in vivo* and moderate energies (80 mJ and 120 mJ at 10 pps) *in vitro* has been demonstrated (Cobb *et al.*, 1992; Whitters *et al.*, 1994). Yet, the bactericidal effect of laser at low energies *in vivo* remains to be elucidated. Moreover, it has not been investigated whether the laser bacterial kill is followed by a clinical benefit. The bactericidal effect, if not associated with destructive effects, may eventually have a clinical benefit. Considering all the reports reviewed in this chapter indicating the potential damage to the biocompatibility of the root surface and pulp at higher energies of the dental Nd:YAG laser, it seems reasonable to focus research on the efficacy and effects of moderate and low laser dosages in periodontal treatment.

1.11 Aims

This thesis attempts to evaluate two of the most recent alternative or adjunctive treatment modalities to root planing developed for periodontal therapy. These are local drug delivery systems and laser therapy.

The main aim of the clinical study of subgingivally delivered antimicrobials was to determine the efficacy of 3 commercially available in the UK locally delivered systems as adjuncts to scaling and root planing in refractory periodontitis sites with a poor response to previous mechanical treatments. Comparisons were made with scaling and root planing alone as the traditional treatment. A further aim was to determine the safety and potential side effects of these systems. Using multivariate statistical analytical methods, the study also sought to investigate the effect of cigarette smoking on the periodontal treatment outcome.

The aims of the *in vitro* study on the Nd:YAG laser were: 1) To determine whether the Nd:YAG laser energy could selectively ablate calculus without affecting underlying cementum and dentine; 2) To determine whether the ablative effect of a given laser energy dosage on the calculus and root surface, is repeatable i.e. whether it could be used in a predictable manner.

The aim of the clinical laser study was to determine whether the low to moderate energy dosages of the Nd:YAG

laser are capable of killing subgingival bacteria and thereby, improving clinical parameters associated with periodontal disease. A further aim was to examine the effect of these laser settings on the root surfaces *in vivo* using the SEM.

CHAPTER II

MATERIALS, METHODS & PRELIMINARY EXPERIMENTS

2.1 Introduction

The intention of this chapter is to describe the materials and methods used in this thesis. The chapter consists of 2 main parts. The first section of the first part describes the measurements of probing pocket depth and attachment level. The next 3 sections describe the preliminary studies related to the study of subgingival antimicrobial systems. These include 1) comparison between 2 pressure sensitive probes, 2) determination of the intra-examiner variability in the measurement of probing pocket depth and attachment levels, and 3) estimation of an approximate sample size for the main clinical study. The fifth section describes in detail the materials and methods used in the clinical study of subgingival antimicrobial systems. The second part of the chapter deals with the laser studies. The preliminary experiments related to the main *in vitro* laser study are described first. These include 1) preliminary evaluation of the application of the Nd:YAG laser using an *in vitro* model, 2) determination of a range of laser parameters, suitable for use in the main *in vitro* study, and 3) evaluation of cracks observed following SEM examination of laser-treated root surface. The materials and methods used in the main *in vitro* laser study are then described in detail. The final section of this chapter describes the materials and methods used in the clinical laser study.

2.2 The clinical study of subgingival antimicrobial systems: preliminary studies, materials and methods

2.2.1 The Florida probe in measurements of probing pocket depth and probing attachment level

The Florida probe (Florida Probe Corporation, Florida, USA) (Gibbs *et al.*, 1988) was used for measuring the probing depth and attachment level in this study. This is an electronic pressure sensitive probe set at 20 g. The Florida probe records the pocket depth and attachment level to the nearest 0.2 mm. The system consists of a pocket depth handpiece and 2 attachment level handpieces (the 'Disk' and the 'Stent' handpieces), a foot switch, computer interface and personal computer (Figs. 2.1, 2.2). The probe tip has a diameter of 0.4 mm, and no visible graduation along its length and it reciprocates through a sleeve. The edge of the sleeve is the reference from which measurements are recorded. A fixed reference point is required for attachment level measurements.

Florida probe 'Stent' handpiece: The Florida Probe 'Stent' handpiece has a 2 mm diameter disc at the edge of its sleeve which, during attachment level measurement, is seated on a custom made soft acrylic stent covering the crowns of the teeth. These stents were constructed for both upper and lower arches for each patient from a 2 mm thick silicone layer (Kombiplast, Dreve-Dentamid) using a

modification of the method described by Isidor, Karring and Attström (1984a). The stents were made on stone models using a vacuum forming machine. The stent was then trimmed to cover approximately the coronal one third of the crowns of the teeth selected for the study. At other teeth, the stent was preserved as much as possible to assist location and retention. Using an inverted cone bur and a low speed handpiece, grooves approximately 1 mm deep, 2 mm wide and 1 mm high were cut, about 1 mm away from the apical edge of the stent, at the study sites. The grooves were then marked with a permanent pen for convenience of identification. The disc of the 'Stent probe' handpiece was located in the base of these grooves during attachment level measurements. Attachment level was assessed relative to the point at which the probe handpiece disc was seated on the stent (Fig. 2.3). In this way, attachment level measurements do not rely on careful location of the cementoenamel junction (CEJ). The identification of the CEJ is often complicated by its subgingival location or the presence of restorations. The grooves on the stent were located at the tooth's maximum height of contour for buccal/lingual measurements, and as close to the contact point as possible for interproximal recordings.

Florida probe 'Disk' handpiece: The Disk handpiece has a disk of 10 mm width and 1 mm thickness which seats on the occlusal/incisal surface of the tooth. The probe tip was

placed into the pocket with the probe tip parallel to the long axis of the tooth. When the disk was brought in contact with the occlusal/incisal point, the foot switch was pressed and the value recorded (Fig. 2.4).

Pocket depth Florida probe handpiece: The pocket depth was recorded when the probe sleeve was brought into contact with the gingival margin without causing blanching of the gingiva due to excessive pressure (Fig. 2.5). Probing depth was also measured at the same points as attachment level recordings i.e., as close as possible to the contact point at the interproximal sites, and at the maximum height of contour at the buccal/lingual sites. The probe tip was kept parallel to the long axis of the tooth during the measurements of attachment level and pocket depth.

Pocket depth and attachment level measurements were measured electronically, and transferred automatically to the computer by pressing the foot switch. Recordings were displayed on the computer screen which was hidden from the operator in order to allow for 'blind' measurements. The measurements were recorded by an assistant.

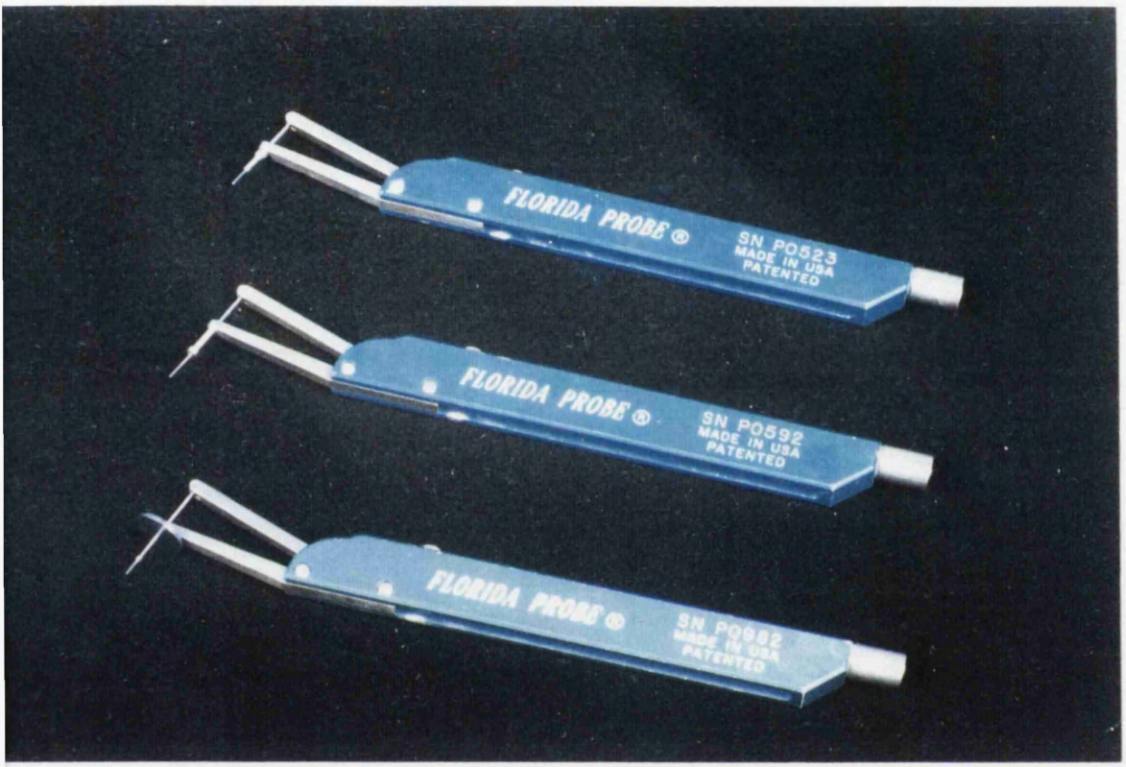

Fig.2.1 The Florida probe; the pocket depth handpiece (top), the 'Stent' attachment handpiece (middle) and the 'Disk' attachment level handpiece (bottom).

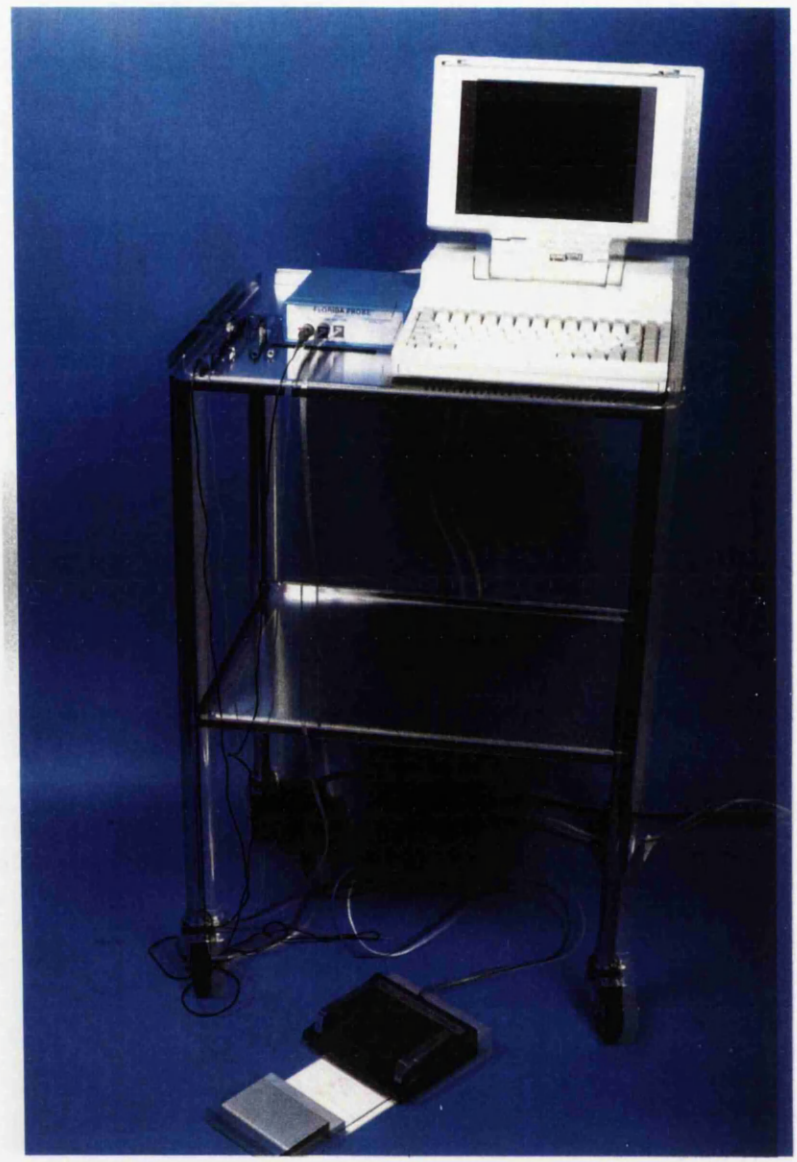

Fig. 22.22 The Florida probe computer interface, monitor and foot switch.

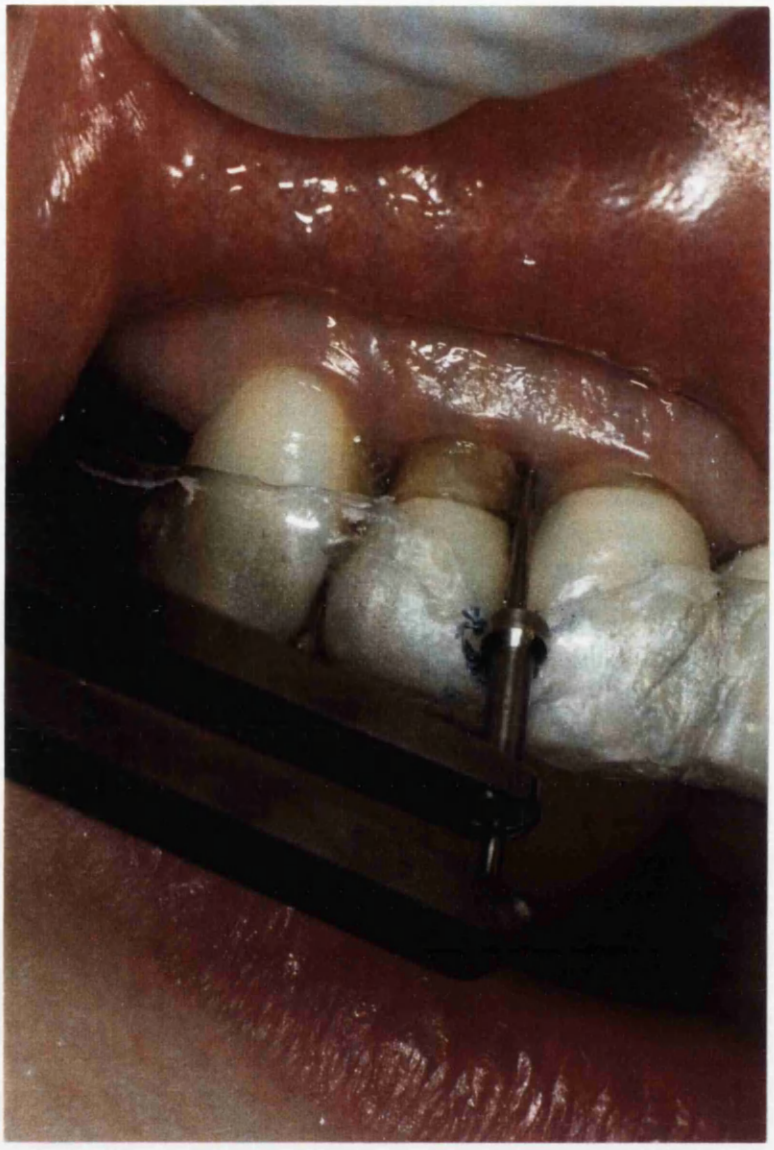

Fig.2.3 'Stent' attachment level handpiece in use with the occlusal stent.

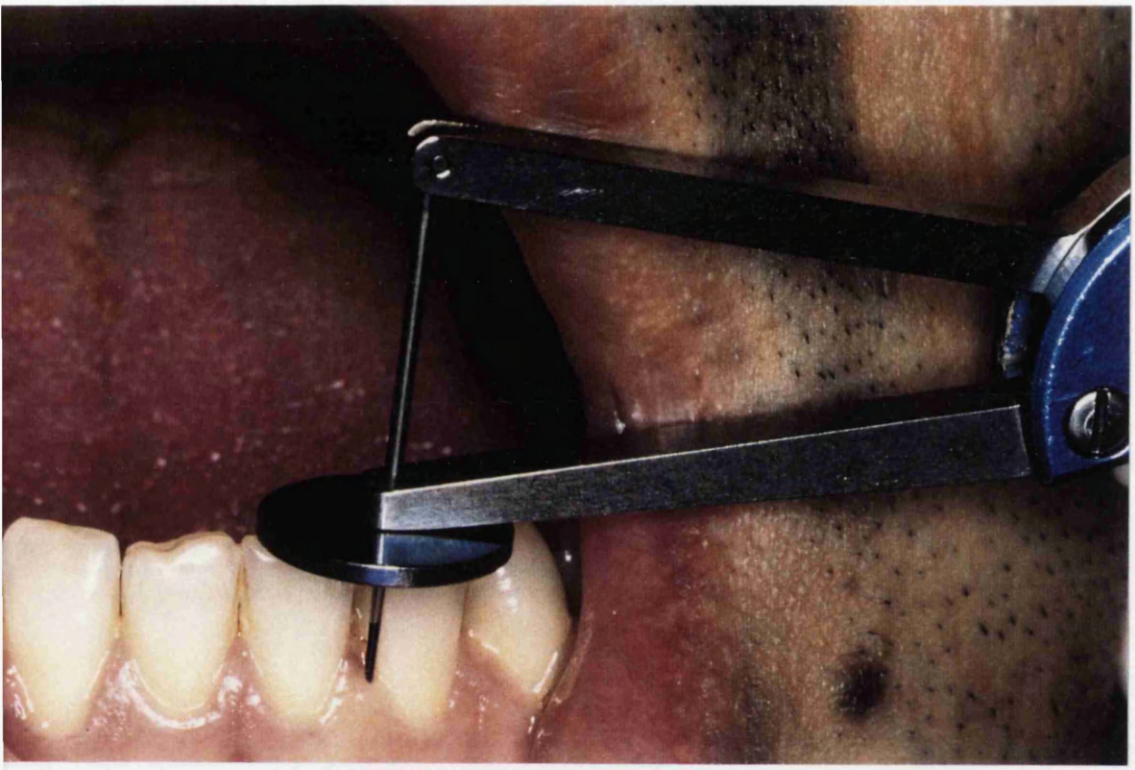

Fig.2.4 'Disk' attachment level handpiece in use.

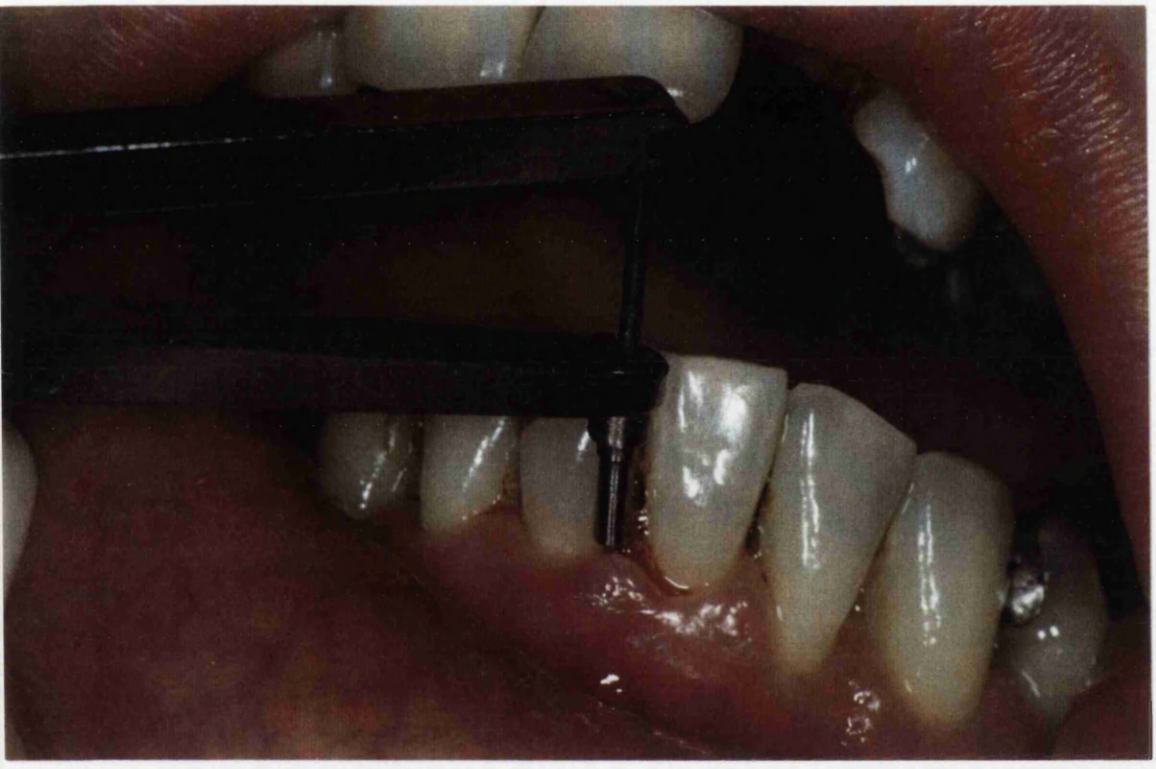

Fig.2.5 The 'Pocket depth' handpiece in use.

2.2.2 Preliminary study; Comparison between two versions of the Florida probe for attachment level measurements

2.2.2.1 Introduction

The reproducibility of the 'Stent probe' and the 'Disk probe' has been compared in pockets < 4 mm (Marks *et al.*, 1991). In their study, the reproducibility of the 'Disk probe' was found to be slightly less than the 'Stent probe', however, there have been no reports with regard to the reproducibility of these 2 probes in deep pockets. In terms of longitudinal attachment level changes, deep periodontal pockets are usually of most interest to clinical researchers. In view of this fact, the need for such a comparison is obvious. Prior to beginning the longitudinal study of subgingival antimicrobial treatment modalities for periodontal disease, it was decided to address the question of which of these 2 probes is preferable. The use of the Stent probe requires the fabrication of an occlusal acrylic onlay. This is time consuming and necessitates some clinical and laboratory effort, moreover, an additional appointment is required. If the Disk probe shows the same degree of reproducibility as the Stent probe, the measurement of attachment level by the Disk probe would be preferred. The aim of this pilot study was to compare the reproducibility of 2 pressure sensitive probes.

2.2.2.2 Material & methods

The study material consisted of 126 sites in 21 patients who were in the maintenance phase of their periodontal therapy. It was decided to select a variety of sites with regard to the degree of gingival inflammation, pocket depth and the state of bleeding on probing. Each patient was seen on a screening visit, sites were selected and impressions were made for stent fabrication. At the second visit, the following parameters were determined by a single operator: the Modified Gingival Index (Loebene *et al.*, 1986)(described in section 2.2.5.4); the presence or absence of bleeding on probing up to 30 seconds after probing with the pressure sensitive probe; attachment level and probing depth using duplicate measurements.

Attachment levels were measured twice using both the 'Stent probe' and the 'Disk probe'. The sequence of measurements was: 1) Stent probe; 2) Disk probe; 3) Stent probe; and 4) Disk probe. The interval between measurements with the same probe was 20 minutes. The difference between the first and the second measurement was computed for each probe and the standard deviation of the differences were calculated.

2.2.2.3 Results

The standard deviation of 126 differences between the first and second measurements of attachment levels using the Stent probe and the Disk probe were 0.600 and 0.676

respectively. Using both probes, the reproducibility of the sites with pocket depth of ≥4mm, sites with bleeding on probing, and sites with GI ≥2 were lower than sites with pocket depth <4mm, sites without bleeding on probing and sites with GI<2 (Table 2.1).

2.2.2.4 Discussion

In this pilot study, the reproducibility of the Stent Florida probe and the Disk Florida probes were compared. In the longitudinal studies of periodontal disease, where change in attachment levels is a major criteria in distinguishing between active sites and inactive sites, or successfully treated and unsuccessfully treated sites, it is essential to use methods which minimise measurement errors. It was found in this study that the standard deviation of Stent probe measurements was narrower than the Disk probe measurements. This finding warrants the use of the Stent rather than the Disk probe in the longitudinal studies of periodontal disease.

One may argue that the acrylic stents may lose their close fit to the occlusal plane over time. Nevertheless, the personal experience of the author is that if the stents are kept on the stone models, warpage will be minimal. However, if the teeth move during the 2 measurement periods, the stent should be cut to perfectly fit on the tooth to be measured. *In vitro* models should be used to precisely evaluate the effect of slight tooth movements on

able 2.1 The difference between the duplicate recordings nd standard deviation obtained using the Disk probe and the tent probe.

Sites	n	Stent probe		Disk probe	
		mean difference	Standard deviation	mean difference	Standard deviation
ll sites	126	-0.011	0.600	-0.016	0.676
D ≥ 4mm	72	-0.019	0.684	0.022	0.721
D < 4mm	54	-0.000	0.471	-0.067	0.614
I ≥ 2	70	-0.043	0.613	0.040	0.736
I < 2	56	0.029	0.586	-0.086	0.592
OP (+)	62	-0.065	0.644	0.006	0.756
OP (-)	64	0.041	0.555	-0.037	0.594

the reproducibility of the attachment measurements using an acrylic flexible stent. Nevertheless, the slight changes in the position of teeth may also affect the position of the disk of the Disk probe in relation to the occlusal surface or incisal edge. The slight superiority of the Stent probe to the Disk probe may be explained by the fact that the stent could provide a reference point for the investigator in both corono-apical and mesio-distal dimensions, whereas the disk could provide a reference point only in the corono-apical direction and an error in the horizontal placement of the probe tip is then more likely to occur.

The only previous report on the comparison of the Stent and the Disk probes (Marks *et al.*, 1991) was consistent with the findings of the present study, although in the study of Marks *et al.* the sites had mild periodontal problems with shallow pockets.

The findings of higher reproducibility in shallower pockets with low gingival inflammation in this study are consistent with previous studies (Badersten *et al.*, 1984a, Isidor *et al.*, 1984a).

A longitudinal study using both probes is required to draw more precise conclusions about the ability of the 2 probes to detect attachment level changes.

2.2.3 Preliminary study; The intra-examiner reliability of duplicated measurements of probing depth and attachment levels.

2.2.3.1 Introduction and methods

In order to improve the reproducibility of the measurements, it was decided to take duplicate measurements rather than single ones. The standard deviation of differences between the first and second readings would provide a measure of the reproducibility and could be used to define a threshold for changes in probing pocket depth or attachment levels. Before the beginning of the clinical study of subgingival antimicrobials, the reproducibility of the examiner for the duplicate measurements of pocket depth and attachment level was determined.

Forty two sites from 7 patients were selected for the study. Each patient was examined 4 times by a single examiner using the Probing Depth Florida probe and 4 times using the Stent Florida probe. There was a 20 minute interval between each series of duplicate recordings. The recordings were displayed on a computer monitor which was hidden from the examiner and were recorded by an assistant. The average of the first and second readings was considered as the first duplicate and the average of the third and the fourth measurements was considered as the second duplicate. The intra-examiner variability of

these 2 measurements would provide a measure of the reproducibility of the duplicate measurements.

2.2.3.2 Results and Discussion

The standard deviations of single and duplicate differences for probing depth were 0.60 and 0.42 respectively and the standard deviations of single and duplicate differences for attachment levels were 0.60 and 0.34 respectively (Table 2.2).

The correlation coefficients between the first and second readings are shown in Table 2.3. The coefficients of duplicate probings are higher than the coefficients of single probings. This held true for both probing depth and attachment level measurements.

Both pocket depth measurement and attachment level measurements showed a marked improvement in reproducibility when duplicate versus single readings were taken. This improvement is more pronounced in attachment level measurements than pocket depth measurements. This finding is in close agreement with Osborn *et al.*, (1990) who found marked advantage in duplicate versus single measurements of attachment level using the Florida probe, but found little advantage in duplicate measurements over single measurements of probing depth using the Florida probe.

Table 2.2 Mean and standard deviations of single and duplicate differences of probing depth and attachment level measurements.

	n (differences)	mean difference	Standard deviation
Probing depth: (mm)			
Duplicated pair per site	42	-0.16	0.42
Single pair per site	42	-0.17	0.60
Attachment level: (mm)			
Duplicated pair per site	42	-0.01	0.34
Single pair per site	42	0.03	0.60

Table 2.3 Correlation coefficients for probing depth and attachment level measurements using single and duplicate recordings.

	Single pairs	Duplicate pairs
Pocket depth	0.93	0.96
Attachment level	0.95	0.97

One may argue that the application of 4 consecutive probings to one site may lead to bias in the measurements or it may have a deteriorating effect on the site. This study showed that there is only a minor tendency towards deeper measurements in the second readings when compared with the first ones (Table 2.2). Moreover, in view of the low pressure applied by the Florida probe compared with manual probes, the repeated probing does not seem to warrant any concern.

In conclusion, it seems that the reliability of the measurements will improve markedly if one uses duplicate probing depth and attachment level recordings instead of single ones. Therefore, duplicate recording was chosen as the method of data collection for these 2 parameters in the main study.

2.2.4 Preliminary study; The estimation of sample size for the study of subgingival antimicrobial therapy

2.2.4.1 Introduction

Conducting a clinical trial is often time consuming and practical obstacles such as time and finance, force the investigators to estimate the minimal size of the required sample prior to the start of the study. The results of the sample size estimation may also convince the

investigator not to start the study, if the required size is too large. To determine the size of sample in a comparative trial, the investigator should have some primary information about the data which he will collect. If the study aims to compare 2 treatments using a quantifiable variable, firstly, the magnitude of the difference in the response variable should be known, and secondly, the variance of this difference should be known. There are only 2 ways to find out these 2 elements:

1) If there has been any previous similar research, the obtained mean difference and the standard deviation of the differences between 2 groups may be used to estimate the sample size. This approach is often too crude and impractical because of the large variability in the methods of data collection, instruments, patient populations and the reproducibility of investigators among different centres. Moreover, a previous direct comparison between the 2 treatments of interest to the investigator, often does not exist.

2) The other approach is to carry out a pilot study of exactly the same design as the main study but at a smaller scale to estimate the mean difference and standard deviation. This approach is also crude due to the use of too few samples. The estimated size is, thus, only a crude guess based on the few available data. In the absence of any similar information about the distribution and the

magnitude of the real difference between the efficacy of the treatments, this would be the only way of estimating the size of a sample, with a certain likelihood of demonstrating a statistically significant difference between the treatments. This latter approach was attempted in this pilot study.

2.2.4.2 Material & methods

Nine patients in the maintenance phase of periodontal treatment, with 4 pockets were screened, selected and randomly assigned to one of the following groups:

1) Scaling and root planing plus application of 25% tetracycline fibres.
2) Scaling and root planing plus application of 2% minocycline gel.
3) Scaling and root planing plus application of 25% metronidazole gel.

Probing pocket depth measurements were obtained at baseline and 6 weeks after treatment using duplicate recordings with the Florida probe. The detailed clinical treatment methods and measurements are described in the section 2.2.5.

Based on the mean pocket depth reduction for each individual, the sample sizes were determined at 3 different power levels i.e. 80%, 90% and 95%. β is the

probability of making a type II error in the statistical inference. This is the probability of accepting the null hypothesis while it is false. Significance level was set at 1%, 5% and 10% levels. The significance level is the probability of making a type I error which is the probability of rejecting the null hypothesis while it is true. The sample size was estimated using the formula (Armitage and Berry, 1987):

$$N > 2 \left\{ \frac{(Z_{2\alpha} + Z_{2\beta})^2 \sigma^2}{\delta_0^2} \right\}$$

When $Z_{2\alpha}$ and $Z_{2\beta}$ represent the values which are exceeded by a standard normal random variable with probability of α and β respectively. δ_0 is the mean difference improvement between the 2 groups and finally, σ is the standard deviation of the difference in the response variable i.e. pocket depth reduction between the 2 groups.

Since, sites with initially deeper pockets usually show higher improvements after treatment as compared with shallower pockets, the baseline pocket depth data was used as a covariate and the differences were adjusted with the covariates. An analysis of covariance was used to calculate the covariate-adjusted pocket depth reductions in 3 groups. The squared root of the error term mean-square in the analysis of covariance output table, was the standard deviation (σ) of the covariate-adjusted pocket depth changes in the sample. The difference between the

covariate-adjusted changes in the 2 groups with the best and second best results is the (δ_0).

2.2.4.3 Results

The results of the study after 6 weeks are shown in Table 2.4. The largest reduction in pocket depth was obtained in the patients treated with scaling plus tetracycline fibres, with a mean pocket depth reduction of 1.80 mm. In the other 2 groups the reductions were 0.483 mm and 0.742 mm for the minocycline gel and the metronidazole gel respectively. The 2 highest reductions were obtained with the tetracycline fibre and the metronidazole gel treatments respectively. The analysis of variance and the Tukey multiple comparisons showed that there was no statistically significant difference among the treatments with 3 subjects per leg (Tables 2.5 and 2.6). The required sample size using the absolute mean values at different probabilities of making the type I and type II errors are shown in Table 2.7. To demonstrate that the tetracycline fibre is superior to both the metronidazole gel and the minocycline gel using absolute mean differences (Delta = 1.07, SD= 0.717), the size of the required sample would be 8 subjects per group if $1-\beta = 80\%$ is accepted at probability level of p=5%. Using the $1-\beta = 90\%$ the sample size would be 11 subjects per group.

When the effect of the baseline pocket depths were taken into consideration as a covariate, the adjusted pocket

Table 2.4 Baseline probing depths and their reductions for the 3 treatments based on subject means (pilot study).

Treatment	n	mean PD baseline	mean PD reduction	SD
Scaling & tetracycline fibre	3	5.64	1.81	0.80
Scaling & minocycline gel	3	5.02	0.48	0.21
Scaling & metronidazole gel	3	5.03	0.74	0.93
Pooled	9	5.23	1.01	0.72

PD, Pocket depth

SD, Standard deviation

Table 2.5 Analysis of variance for differences between treatments.

source	S.S.	D.F.	M.S.	F	*p*
Between groups	2.95	2	2.48	2.88	0.133
Within groups	3.08	6	0.51		

S.S.,Sum of squares

D.F., Degrees of freedom

M.S., Mean squares

Table 2.6 Matrix of pair-wise comparison probabilities using the Tukey test (pilot study).

	Scaling & tetracycline fibre	**Scaling & minocycline gel**	**Scaling & metronidazole gel**
Scaling & tetracycline fibre	1.000		
Scaling & minocycline gel	0.138	0	
Scaling & metronidazole gel	0.241	0.900	1.000

Table 2.7 The estimated sample size per group using different alpha and beta levels based on the unadjusted data.

1-β	**α=0.01**	**α=0.05**	**α=0.10**
0.8	12	8	7
0.9	15	11	9
0.95	17	13	11

Table 2.8 Unadjusted and covariate-adjusted mean pocket depth reductions among the 3 groups.

Treatment	**Mean PD reduction**	**Covariate-adjusted mean PD reduction**	**Standard deviation**
Scaling & tetracycline fibre	1.81	1.58	0.80
Scaling & minocycline gel	0.48	0.60	0.21
Scaling & metronidazole gel	0.74	0.86	0.93

Table 2.9 Matrix of pairwise covariate adjusted mean differences (pilot study).

	Scaling & tetracycline fibre	Scaling & minocycline gel	Scaling & metronidazole gel
Scaling & tetracycline fibre	0		
Scaling & minocycline gel	0.976	0	
Scaling & metronidazole gel	0.722	0.254	0

Table 2.10 Analysis of covariance on the mean pocket depth reductions with the baseline pocket depths as covariate (pilot study).

source	S.S.	D.F.	M.S.	F	*p*
Treatment	1.263	2	0.632	1.544	0.300
PD0	1.037	1	1.037	2.535	0.172
Error	2.045	5	0.409		

S.S., Sum of squares

D.F., Degrees of freedom

M.S., Mean squares

PD0, Baseline pocket depth

Table 2.11 The estimated sample size per group to demonstrate a significant difference between the tetracycline fibre and other treatments at different α and β levels using the covariate adjusted data.

1-β	α=0.01	α=0.05	α=0.10
0.8	19	13	11
0.9	24	18	15
0.95	29	21	18

depth reductions were 1.58 mm, 0.6 mm and 0.86 mm for the tetracycline fibre, the minocycline gel and the metronidazole gel treatments respectively (Table 2.8). The pooled standard deviation was 0.64. Table 2.9 shows the matrix of pairwise covariate adjusted mean differences. Table 2.10 shows the analysis of covariance on the effect of the 3 treatments with the baseline pocket depth as the covariate. The effect of high baseline pocket depth was stronger than the treatment effect. The sample size required to demonstrate significant differences between groups at different α and β levels are shown in the Table 2.11. At the β-1 = 80% and the α = 5%, the required sample size would be 13 subjects per group.

2.2.4.4 Discussion

The results of this pilot study should be looked at with care because of the relative crudeness of the estimation. It was the assumption of the pilot study that in the main study, pocket depth reductions among the groups would be of the same magnitude and variability as were found in this study. However, in view of the large variability often found between individuals in the response to periodontal treatment, it was not known whether the results of this study would be repeated in the main study. Part of the variability is due to the relatively large measurement errors in the clinical examination of pocket depth and attachment levels. Another part of the

variation is due to biologic variation in terms of the response to the treatment. There is a relatively large variation between sites and individuals in their response to treatment. The subject was considered as the unit of analysis, to take into consideration the 'within-individual' biological dependence, under the influence of systemic factors. This increased the estimated number of required subjects. If the site could have been used as the unit of analysis, the sample size requirements would be relaxed and a smaller number of subjects would be required. In the main study, if a significant difference is detected on the subject analysis, most likely a significant difference would be detected at a site-based analysis too.

In this pilot study it was estimated that a study of 13 subjects in each group i.e. 52 patients with a total of 208 diseased sites should provide sufficient power (80%) to demonstrate a significant advantage ($p<0.05$) of tetracycline fibre therapy in a 4-leg study, assuming it is of the same magnitude as that demonstrated in the pilot study. The probability of making a type II error i.e. β is usually taken as 4 times a. Therefore, at a significance level of $\alpha=0.05$, the $\beta=0.20$ or $1-\beta=80\%$ should be selected (Armitage and Berry, 1987). Obviously, any increase in the value of $1-\beta$ would further increase the power of the study to avoid the type II error.

In this pilot study no control group was used. While no published data is available on the combination of metronidazole gel and scaling compared to scaling alone, some previous studies on the tetracycline fibre and minocycline gel indicated that the combination of scaling with either of them results in a better response than scaling alone (Newman *et al.*, 1994, van Steenberghe *et al.*, 1993). It was assumed that the effect of scaling and root planing alone would not exceed any of the combined treatments. If this assumption was not true, then the required sample size may have been underestimated.

In this pilot study, the analysis of covariance was used to consider the effect of difference in the baseline pocket depths on the treatment outcome. The effect of baseline pocket depth was larger than the effect of treatment. This indicates that to reduce the heterogeneity among the study population, the inclusion of subjects in the study, should result in relatively balanced groups in terms of the baseline pocket depth (pre-stratification). The post-stratification, using the analysis of covariance, will also minimise the remaining inevitable minor differences in the baseline pocket depths.

In conclusion, the target number of subjects would be at least 13 subjects per group i.e. 52 subject. Any excess

number of subjects in each group would add to the statistical power of the study.

2.2.5 Material and methods used in the clinical study of the subgingival antimicrobial systems

2.2.5.1 Clinical design

The study protocol was approved by the local ethical committee. Subjects were selected from among patients attending the Periodontal Unit of the Adult Dental Care Department in Glasgow Dental Hospital for their periodontal treatment. These patients had finished their active phase of therapy and still had periodontal pockets ≥ 5 mm with bleeding on probing. The number of subjects were estimated by a pilot study (section 2.2.4). The minimum target number was 13 patients in each group i.e., a total of 52 patients.

Each patient was to be examined at baseline, 6 weeks, 3 months and 6 months after treatment. After baseline measurements patients were allocated randomly to one of the 4 treatments including: scaling and root planing plus insertion of 25% tetracycline monolithic fibres (Actisite, ALZA corporation, Palo Alto, CA), scaling and root planing plus application of 2% minocycline gel (Dentomycin, Cyanamide of Great Britain, Lederle, Gosport, Hampshire, England), scaling and root planing plus application of 25% metronidazole gel (Elyzol, Dumex Ltd, Copenhagen), and

scaling and root planing alone. The follow-up time points were 6 weeks, 3 months and 6 months after the last visit of treatment.

2.2.5.1 Patient screening and selection

Selection of sites was carried out on a screening visit, consisting of a full-mouth periodontal examination using a PC-12 probe. The patients were required to have periodontal pockets ≥ 5 mm with bleeding on probing or suppuration in at least 4 non-adjacent teeth which either did not respond favourably to the mechanical therapy or showed a relapse of disease in spite of transient favourable response. Sites with furcation lesions were not selected due to their unpredictable response to treatment. Anterior flat surface sites were preferred to the posterior flat sites because of the ease of measurements and treatment and the higher reproducibility in anterior sites (Badersten *et al.*, 1984a; Mullally and Linden, 1994). These patients had maintained a relatively good oral hygiene throughout previous treatment visits as documented in their hospital records.

The patients should not have had any antibiotic treatment during the previous 6 months. Patients with a history of allergy or adverse reaction following the use of tetracycline, minocycline and metronidazole were excluded. Patients with systemic disease, pregnancy, lactation, and a history of oral candidiasis were not selected. Only

patients of at least 30 years of age were selected. By doing so, subjects with localised juvenile periodontitis would not be selected and a more homogenous population would be included in the study. If the patient fulfilled the inclusion criteria, informed consent was obtained. Alginate impressions were made at the same or subsequent visit for the fabrication of the measurement stents. Oral hygiene instruction was given according to individual needs.

2.2.5.3 Clinical indices

All the clinical examinations were carried out by a single examiner throughout the whole period of study (MR). The sequence of the measurements was as described below.

2.2.5.4 The Modified Gingival Index

To assess the degree of gingival inflammation at each site the modified gingival index (Lobene *et al.*, 1986) was used by the examiner. The criteria for the modified gingival index are:

Score 0: Absence of inflammation.

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

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

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

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

In this index, unlike other commonly used gingival indices, the bleeding on probing component is not assessed. This allows for a non-invasive assessment of the gingival inflammation by visual examination. Since gingival crevicular fluid (GCF) samples were to be taken from the sites to determine the GCF volume, it was important to use an index with minimal potential for disruption of the GCF volume and its biochemical profile.

2.2.5.5 The Plaque Index

The plaque index of (Silness and Löe, 1964) was used to score plaque accumulation at each site. Each site was dried using a blast of air and a score allocated from a range of 0 - 3. The criteria for plaque index includes:

Score 0: No plaque.

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

Score 2: Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye.
Score 3: Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

2.2.5.6 Gingival crevicular fluid measurements

Gingival crevicular fluid (GCF) volume was measured as an additional index of gingival inflammation. Whatman grade 4 filter paper (Whatman Labsales Ltd., Maidstone, Kent) was cut to 2x13mm strips using a ruler and a scalpel. A line was drawn on each strip at 8 mm indicating the length of the paper strip to be inserted between the jaws of Periotron (Periotron 6000, Harco Electronics, Winnipeg, Canada). Autoclaved paper strips were used for GCF sampling. The area was isolated from saliva contamination with cotton wool rolls and saliva ejector and the gingival site was gently air dried and supragingival plaque removed. The sterilised paper strip was introduced into the crevice until mild resistance was felt and left for 30 seconds. The strip was immediately transferred to the Periotron and the Periotron reading recorded. The jaws of the Periotron were wiped with pure methanol and dried with filter paper between readings (Figs. 2.6 and 2.7).

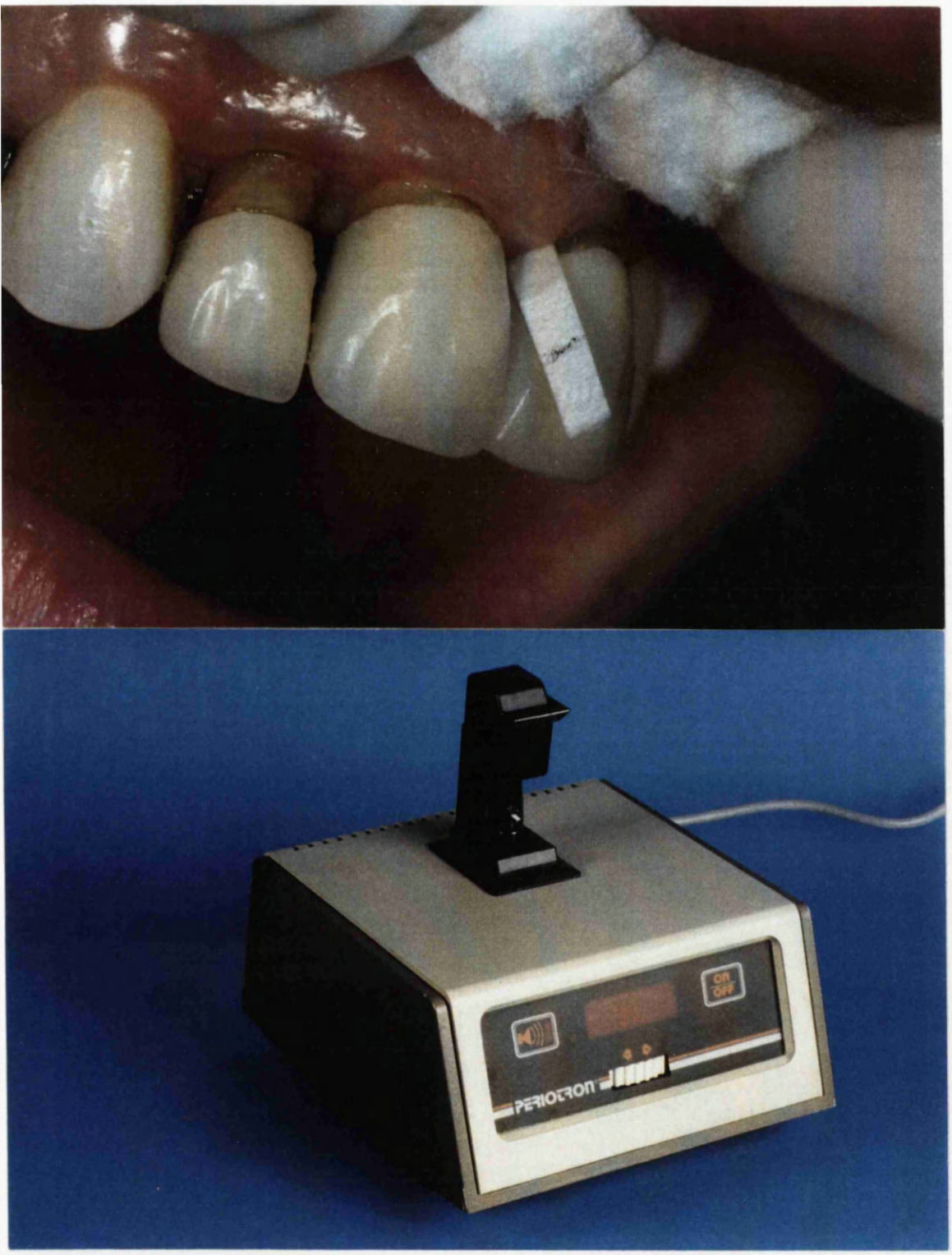

Fig.2.6 Filter paper strips *in situ* (top).
Fig.2.7 Periotron 6000 (bottom).

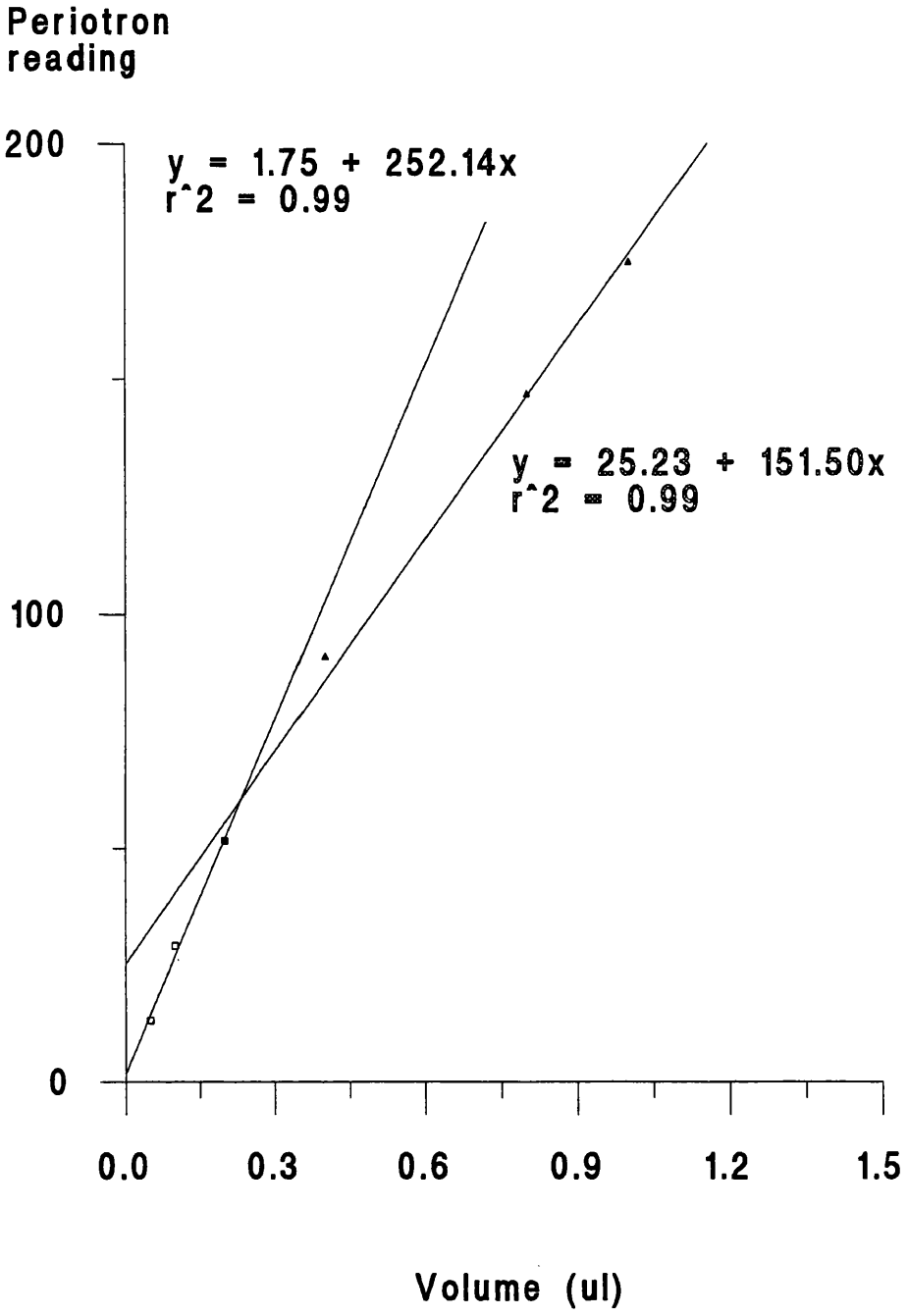

Fig.2.8 Regression lines and equations for the Periotron calibration.

2.2.5.7 Calibration of Periotron

Prior to each visit, calibration of the Periotron was performed. Known volumes of serum diluted 1:1 with phosphate buffered saline were introduced onto paper strips using a Hamilton microsyringe. Volumes included 0.05, 0.1, 0.2, 0.4, 0.8, 1 µl. Each measurement was performed three times and the mean values of each volume were subjected to regression analysis. Two regression lines were constructed: one line for the volumes 0.05-0.20 µl and another line for the volumes 0.20-1.00 µl. The slope and intercept of the regression lines were used to determine the GCF volume (Fig.2.8). The calibration reduced any variation in Periotron measurements due to changes in atmospheric humidity.

2.2.5.8 The probing pocket depth and probing attachment levels

The Florida probe was used for measuring the probing depth and attachment level in this study. Based on the results of our pilot study (section 2.2.2), the use of 'Stent' handpiece was preferred. The Florida probe and the methods of measurements were described earlier in detail (section 2.2.1).

Both recordings were taken in duplicate. First, the first pair of the pocket depth readings was recorded, then the stents were placed and the first pair of the attachment level readings was taken. After removing the stents and

changing the position of the patient, the examiner left the room and 5 minutes later, the same procedures were repeated to record the second pairs of the probing depth and attachment level measurements. The average of the first and second measurements was calculated as the value of the pocket depth and attachment level for that site at that time point. The examiner's reliability in these procedures was determined and described in section 2.2.3.

2.2.5.9 Bleeding on probing (BOP)

Bleeding on probing was scored dichotomously using the Florida Probe (pocket depth handpiece) at the same time as the recording of the first measurement of pocket depth. The presence or absence of bleeding within 30 seconds of probing was recorded.

2.2.5.10 Suppuration

The presence or absence of suppuration was examined by gently applying a ball burnisher on the gingival mucosa in an apico-coronal direction.

2.2.5.11 Controlled randomisation; criteria for patient allocation to treatments

The purpose of patient allocation was to have four groups of patients which were balanced in terms of age and smoking status. The participants in the study were randomly allocated by the therapist to receive one of the treatments. Effort was made to prevent serious imbalance

between groups in terms of the average scores of the above mentioned parameters. While pre-stratification by such a method is not always perfectly feasible, especially if more than one criterion is used for stratification, it can prevent serious imbalance in factors which are known or suspected to influence the treatment outcome.

2.2.5.12 Treatments

All the treatments were performed by a single operator (NP) who had been trained in the use of the antimicrobial systems. In applying the antimicrobial systems, the manufacturer's recommendations were strictly adhered to. The treatments included:

1) Subgingival scaling and root planing under local anaesthesia. All deep pockets within the mouth were treated. Each tooth was instrumented for approximately 5 minutes. Normally, a 60-minute visit was required for this purpose. An additional appointment was used if this was required.

2) Subgingival scaling and root planing under local anaesthesia and the application of 25% tetracycline impregnated fibres into the pocket. The fibre delivery system was 0.5 mm diameter ethylene vinyl acetate (EVA) copolymer fibre loaded 25% by weight with tetracycline hydrochloride resulting in a drug concentration of 0.55 mg/cm fibre. The fibre was wrapped loosely around the

teeth by passing it through the contact points using dental floss, a floss threader or a plastic instrument. The pocket was occupied by fibre without creating excessive tension in the soft tissues. A cyanoacrylate adhesive (Octyldent, Tri-point Medical LP, Raleigh, NC) was applied over the gingival crevice to fix the fibre into the pocket and a periodontal dressing (Coe-pak, GC America INC, Chicago, IL) was placed (Fig. 2.9). The fibre was removed 10 days later using periodontal curettes.

3) Subgingival scaling and root planing plus the application of 2% minocycline dental gel to the pocket until it was overfilled. This is a 2% weight by weight minocycline hydrochloride preparation which is presented in disposable plastic applicators, each containing 0.5 g dental gel. The sealed foil packages, each containing one applicator were stored in a refrigerator (2-8° C) and were allowed to equilibrate to room temperature 15 minutes before application. Between application from site to site within the patient, the applicator nozzle was cleaned with a disposable alcohol impregnated tissue (Azowipe, Vernoncarus, England). Gel application was repeated 14 days and 28 days after the initial application (Fig. 2.10).

4) Subgingival scaling and root planing plus the application of 25% metronidazole dental gel.

Metronidazole gel is a semisolid suspension containing metronidazole benzoate corresponding to 250 mg metronidazole per gram of gel. It is presented as disposable intraligamentous injection syringes containing 1 g of gel which is injected into the pocket using a blunt needle until it is overfilled. Each package contains 2 syringes and it should be stored at room temperature (20°C). Between applications from site to site, the applicator nozzle was cleaned with a disposable alcohol impregnated tissue. Gel application was repeated 7 days later (Fig. 2.11).

Patients who received gel applications were instructed not to eat or drink for 3 hours post application. Moreover, they were told not to use interdental cleaning devices in the treated sites for the rest of the day. The patients who received metronidazole gel were forbidden to take alcohol for 24 hours post-treatment to prevent disulfiram like reactions (British National Formulary, 1994).

Treatment time was recorded. The length of tetracycline fibre used for each site was also recorded. At each post-treatment visit, the treated sites were examined for any adverse signs related to the treatments and results were recorded. Any reported symptoms were also recorded.

During this study, other sites received conventional maintenance care according to their individual needs. No

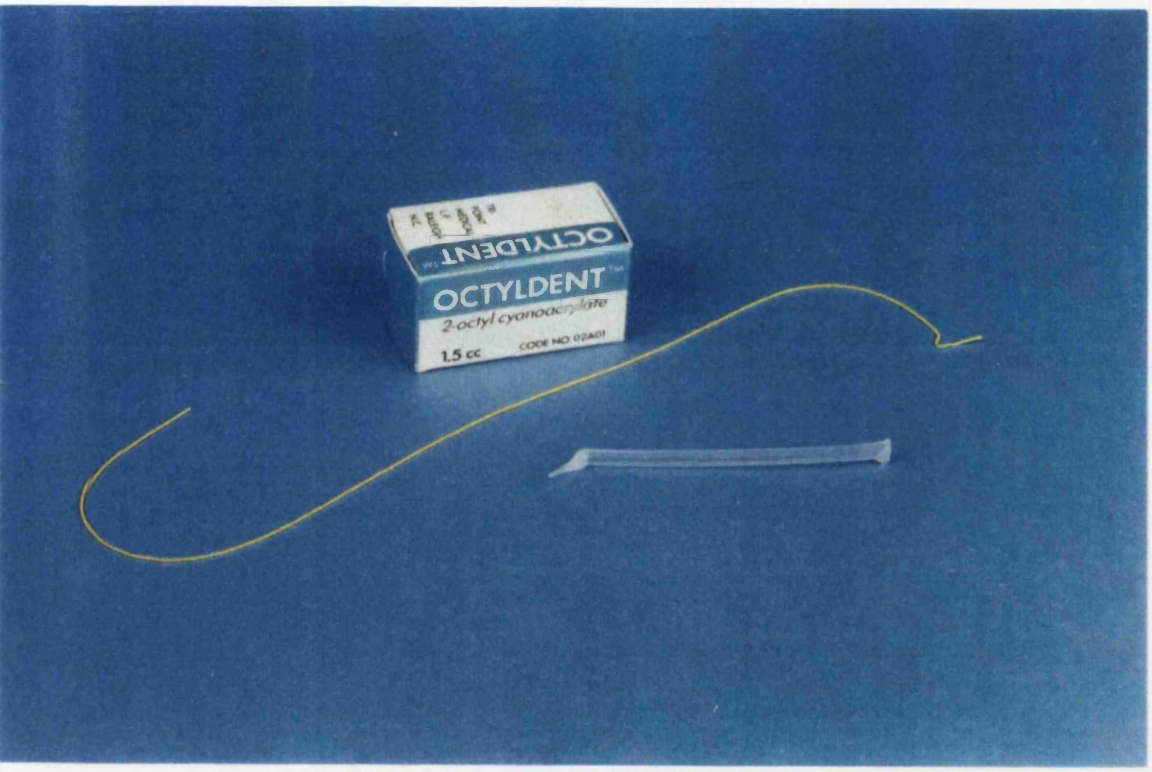

Fig.2.9 Tetracycline fibre, cyanoacrylate adhesive, and the squeezette.

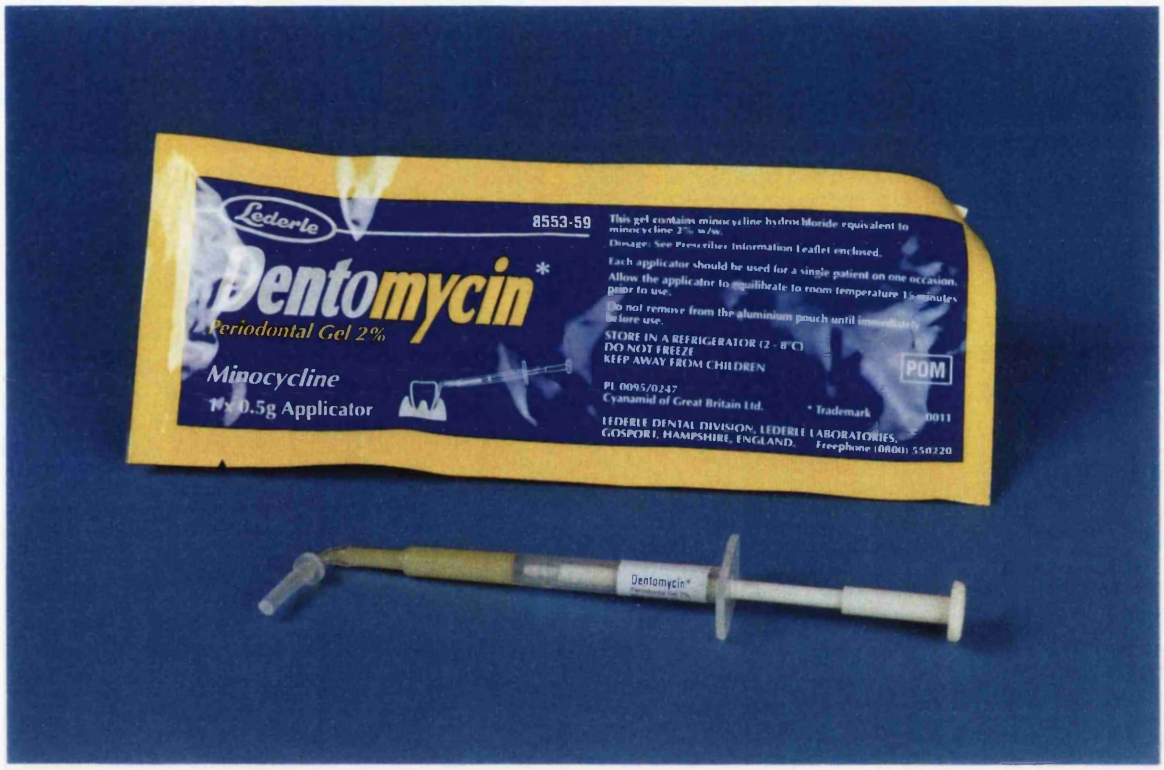

Fig.2.10 Minocycline gel and applicator.

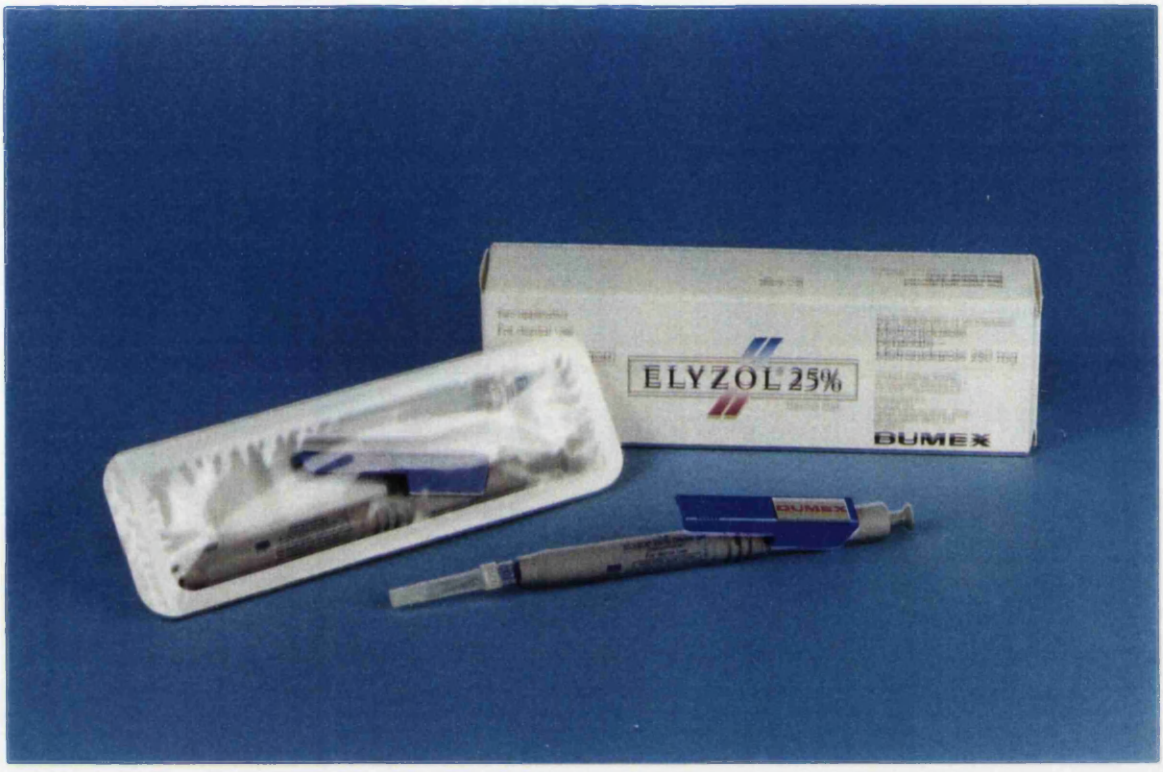

Fig.2.11 Metronidazole gel and applicator.

further treatment was carried out for the study sites during the 6 months follow up period unless a site showed attachment loss more than 2 mm or developed an abscess.

2.2.5.13 Statistical analysis

Pocket depth and attachment level data were analysed on a subject basis, i.e., the values of the 4 sites within each patient were averaged at each time point, to produce a single figure. The significance of the pocket depth and attachment level change from baseline to each follow-up time point within each treatment group was tested using the paired-t test. An analysis of variance using the General Linear Model (GLM) procedures was performed on the change in the pocket depth and attachment levels. The treatment effect was the main factor with 4 levels, and the baseline pocket depth was a continuous covariate. By using this analysis, the effect of baseline pocket depth on the treatment outcome is taken into account. Hypothesis testing was initiated by including the main effect and covariate as well as the interaction between these 2 factors. If the interaction was not significant, the model was tested again without the interaction term. If the *p* value for the treatment effect was significant, post-hoc comparisons were made using the same procedure (GLM), by comparing only 2 treatments at a time, and the significance threshold value was divided by the square root of the number of comparisons (Brown and Swanson-Beck,

1988). Therefore, the significance level was set at $0.05/\sqrt{6}=0.020$.

The significance of change in BOP and suppuration data within each treatment group was tested using the Fisher's exact test for paired data. The chi-square (χ^2) test was used to test the hypothesis of association between the treatments and the improvement in BOP. Since the prerequisites of the chi-square test were not met for the suppuration data due to the presence of small expected counts in too many cells, the Fisher's exact test was used. The MGI scores were subjected to the Wilcoxon's test to examine the significance of change from baseline to each follow-up visit in each treatment group. The Kruskal-Wallis test was used to compare the MGI scores and the PI scores between treatments. If the test was significant, pair-wise comparisons were performed using the Mann-Whitney U test and the p value was adjusted for multiple comparisons. GCF volume data were analysed using the paired-t test for the significance of change within each treatment group, and using the one-way analysis of variance (ANOVA) to compare the change in the GCF volume between groups.

The two sample t-test was used to compare the change in pocket depth and attachment level between smoker subjects and subjects who were not smokers, regardless of the treatment type. To further elucidate the effect of

smoking status on the treatment outcome while considering other important factors, the GLM procedure was used with treatment and smoking status as the main factors with 4 and 2 levels respectively, and baseline pocket depth as a continuous covariate. A backward elimination approach was used to eliminate the interaction terms, that is, the initial model included all 3-order and 2-order interaction terms; the-3-order interaction was eliminated if it was not significant, and the model was tested without the 3-order interaction. The next insignificant interaction term with the largest p value was eliminated and so on. The final model consisted of only individual factors and the significant interaction terms. Since a significant interaction was consistently found between the smoking status and the baseline pocket depth, indicative of heterogeneity of the covariate slopes, between the smokers and non-smokers, linear regression analysis was used for the smokers and non-smokers independently, to examine the relationship between the baseline pocket depth and improvement in pocket depth or attachment level.

2.3 The laser *in vitro* studies

This investigation consisted of 3 preliminary studies, and a main study. The main *in vitro* study aimed to evaluate the effect of the Nd:YAG laser treatment on the subgingival calculus, cementum, and dentine *in vitro*. Prior to the study, 3 pilot studies were performed to determine the methodology of the main *in vitro* study. In the following sections the pilot studies are described subsequently followed by the methods used in the main *in vitro* study.

2.3.1 Preliminary study; Preliminary evaluation of the effect of an Nd:YAG laser on calculus

2.3.1.1 Introduction

This pilot study was used as a preliminary assessment of the effect of laser energy on calculus and root surface. The information obtained from this study and the subsequent pilot studies enabled the investigators to design a suitable method of assessing the laser effects in a semi-quantifiable fashion for the main *in vitro* study. Here, it was intended to mimic the clinical application of the laser using an *in vitro* model. In this study the laser was used either alone or in conjunction with root planing and their effects were compared with root planing alone and no treatment, as positive and negative controls.

2.3.1.2 Materials and methods

Six freshly extracted teeth with subgingival calculus were selected. The coronal half of the roots was covered with dental modelling wax of 2mm diameter. The entire length of the roots were then embedded in dental plaster. When the plaster had set, the wax was removed with warm water, leaving an exposed area of root surface similar to a periodontal pocket (Fig. 2.12). Each of the 4 surfaces of each tooth was randomly assigned to one of the following treatments: A) laser treatment alone, B) root planing alone, C) laser treatment plus root planing and D) untreated control.

One of the following 6 different laser treatments was randomly applied to each tooth: 1) 50 milli-Joules (mJ), 10 pulses per second (pps) for 1 minute (min); 2) 50 mJ, 10 pps for 2 min; 3) 100 mJ, 15 pps for 1 min; 4) 100 mJ, 15 pps for 2 min; 5) 125 mJ, 20 pps for 1 min; and 6) 125 mJ, 20 pps for 2 min. The optical fibre was kept in contact with and parallel to the root surface and the tip of the probe was moved with overlapping strokes to ensure coverage of the entire area being treated.

After treatment, the teeth were gently removed from the surrounding plaster and slabs of approximately 2mm in thickness containing the treated surfaces were cut from the teeth using a handpiece and disc. These were fixed in 10% neutral buffered formalin for 3 hours, then air dried

for 48 hours. The specimens were mounted on 32mm diameter aluminium stubs with Leit C conductive carbon adhesive, sputter coated with gold (Polaron-E 5000) and then examined by a scanning electron microscope (SEM) (Jeol-T300) operating at an accelerating voltage of 30 kV.

2.3.1.3 Results

It was observed that laser treatment alone at any of the pulse energies tested did not remove the calculus from the specimens, but areas of black, charred calculus were observed on specimens treated with the laser energies equal or higher than 100 mJ (Fig. 2.13). At the pulse energy of 50 mJ, no change occurred as compared to the untreated controls.

At higher magnifications under SEM, scattered areas of melted calculus could be seen. However, this pattern was the same for all surfaces treated by laser energies of equal or higher than 100 mJ (Fig 2.14a) and it was not possible to differentiate between the effect of different energy settings.

In some of the specimens in the group treated with the laser plus root planing, the cementum appeared to have peeled away from the root surface, exposing dentinal tubules (Figs. 2.14b,c).

Fig.2.12 Experimental teeth cast in plaster (preliminary study).

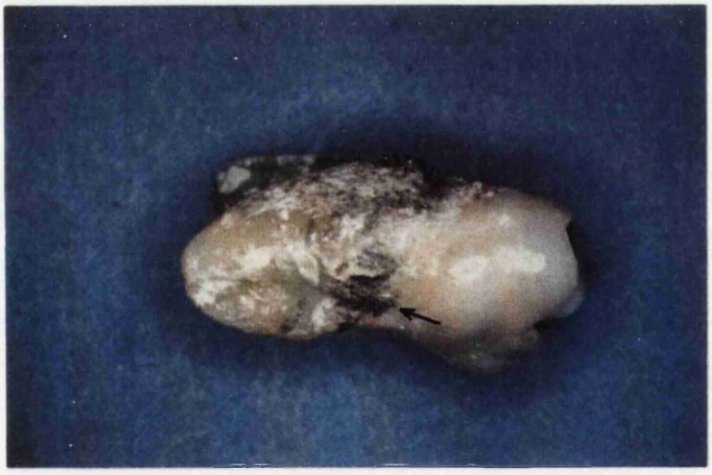

Fig.2.13 Laser treated calculus (125 mJ, 20 pps, 2 minutes). The black coloured area is the charred calculus.

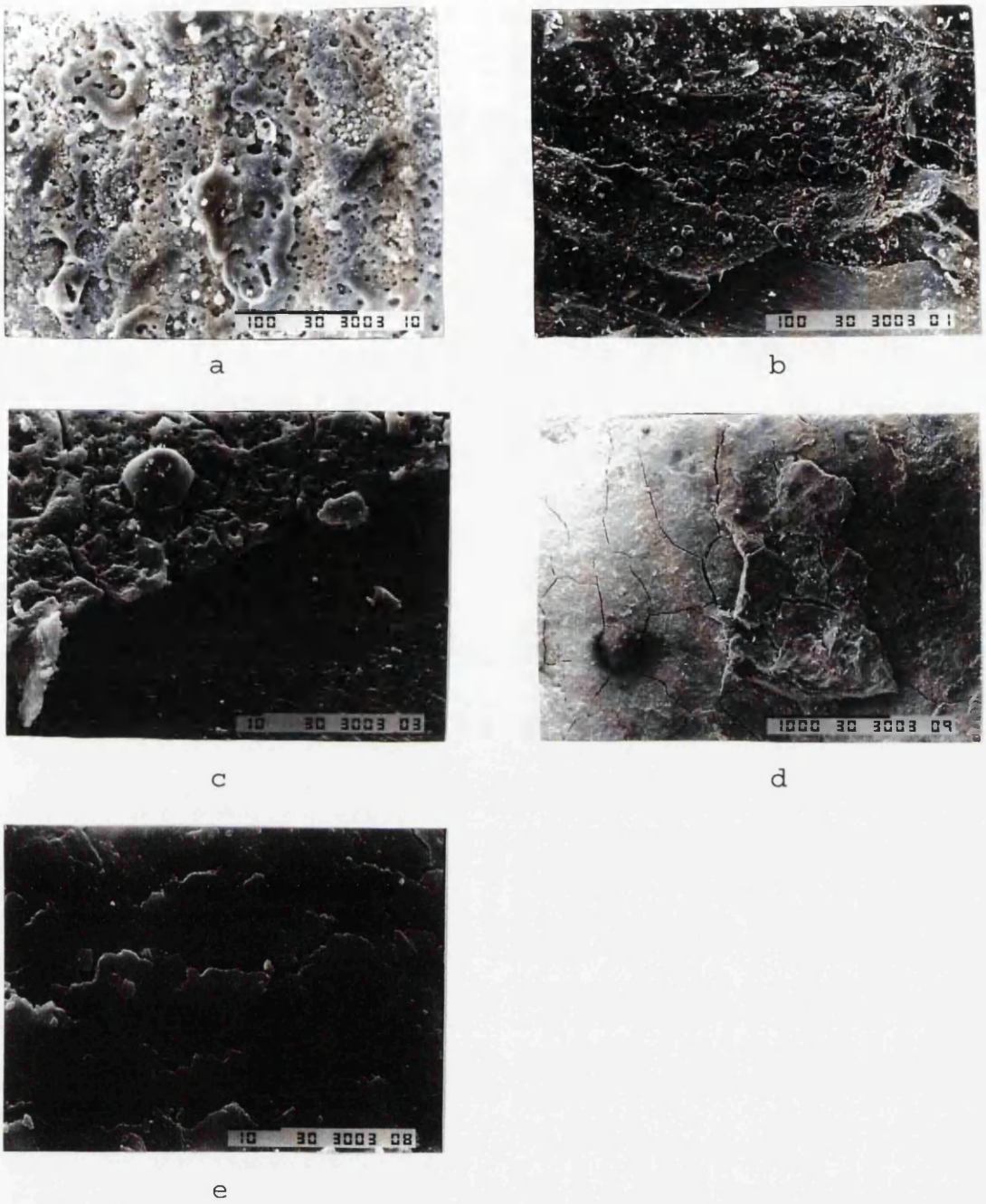

Fig.2.14. SEM micrographs of the laser treated root surface (preliminary study).
a: Laser treated calculus (100 mJ, 15 pps, 2 minutes); The calculus surface has been melted down.
b,c: The laser treatment (125 mJ, 20 pps, 1 minute) followed by root planing on the cementum. The cementum layer has been peeled off after root planing, exposing the dentinal tubules (c).
d: Surface cracking of the specimen (see the text).
e: Cementum surface treated with root planing alone; The surface is covered with a scale like smear layer.

Many cracks were observed on the surface of the cementum and calculus in most of the specimens (Fig. 2.14d). The presence of these cracks made a precise evaluation of the effects of the treatments on the specimens difficult. On surfaces which had only been root planed, a smear layer was observed over most of the treated areas on all specimens (Fig. 2.14-e). However, the treated area was smoother than laser treated surfaces and little measurable amount of calculus was observed.

2.3.1.4 Discussion

In previous studies on the effects of laser treatment on root surfaces (Cobb *et al.*, 1992; Morlock *et al.*, 1992; Trylovich *et al.*, 1992) a protocol similar to the one used in this study was employed, i.e. overlapping movements of the tip of the laser handpiece parallel to the root surface. In view of the fact that the laser beam produced only has a diameter of 320 μm, it is likely that during the limited treatment time, many areas of the specimens may not have been lased, whereas other areas may have been treated more than once. Because of this limitation, the treatment effects could not always be evaluated precisely.

Although this study showed that treatment with the laser beam had a melting effect on the surfaces of the treated specimens, it was not possible to differentiate the effects of different energies. Therefore, in the main experiment, it was decided to keep the fibre stationary and perpendicular to the root surface for the duration of

the treatment period in order to localise the treated area.

The presence of surface cracks on the roots was probably due in part to the rapid evaporation of water in the partial vacuum of the coating chamber. The use of alcohol baths to dehydrate the specimens before drying in air may decrease the number and severity of the cracks on the surface. If the whole tooth was used instead of 2mm thick slabs, the greater bulk of dentine present may be less prone to cracking than a thin specimen. It was decided that the whole tooth should be dehydrated in increasing concentrations of ethanol and finally immersed in hexamethyldisalazane before air drying (Morlock *et al.*, 1992) to reduce the likelihood of cracking. Moreover, the cracking of specimens was addressed in a pilot study.

2.3.2 Preliminary study; Selection of laser settings

2.3.2.1 Introduction and methods

Since the purpose of the main *in vitro* study was to evaluate the effects of different laser settings on calculus, cementum and dentine in terms of physical damage, this preliminary study was carried out to determine which settings appeared to be suitable for SEM study. From this information, a range of laser settings for the main *in vitro* study would be selected.

2.3.2.2 Materials and methods

Two calculus covered surfaces were laser treated. Each tooth received the following laser settings: 30mJ, 50mJ and 100mJ at 10pps; and 100mJ at 20pps, each for 1 and 5 seconds. The handpiece was kept stationary, perpendicular to the surface using an adjustable jig (Fig. 2.15). The specimens were then prepared and examined by SEM. The preparation of specimens for SEM have been described in sections 2.3.4.7-10.

2.3.2.3 Results

SEM examination showed no change in the specimens treated at 30mJ, 10pps for both 1 and 5 seconds. At 50mJ, 10pps for 1 sec, slight changes were noted in one of the specimens, the other did not appear to have been damaged. The same held true for treatment for 1 second at 100mJ, 10pps. Moderate damage was observed on both of the specimens treated for 5 sec at 50mJ, 10pps. Treatment at 100mJ, 10pps for 5 sec and 100mJ, 20pps for both 1 and 5 sec resulted in severe damage to the calculus. The bottom of the pit produced by the laser treatment was hardly visible under the SEM, indicating that the underlying hard tissues were severely damaged.

2.3.2.4 Discussion and conclusion

Moderate damage was produced by treatment with the laser at 50mJ, 10pps for both 1 and 5 sec, and 100mJ, 10pps for 1 sec, yet one of the specimens treated at 50mJ, 10pps for

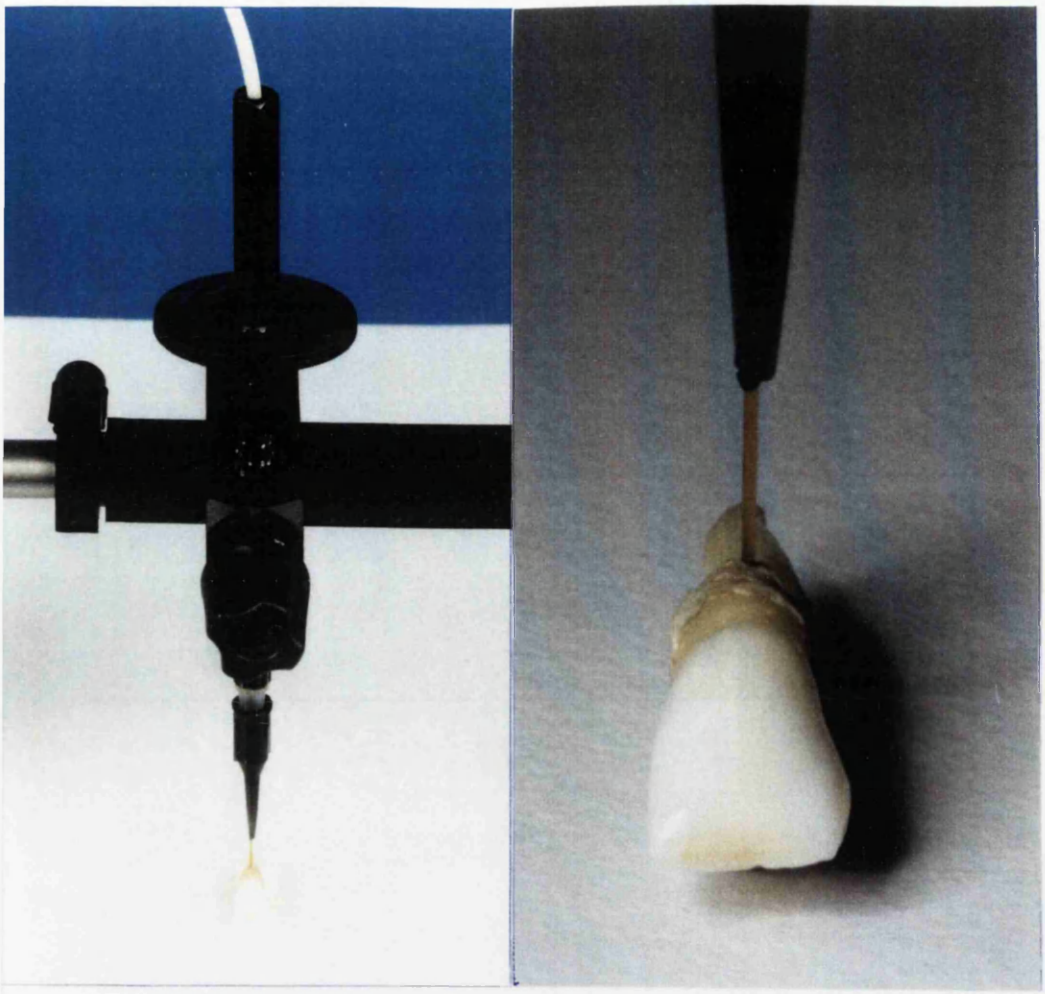

Fig. 2.15 The jig used to stabilise the laser handpiece

1 sec did not appear to be affected. Treatment settings other than these values produced either no visible effect or very severe damage to the cementum and therefore did not seem suitable for further investigation by the SEM. From the results of this preliminary study, values of 50mJ and 100mJ for 1 and 5 seconds duration were chosen for further investigation. Pulse rate settings of 10pps and 20pps were also included in the protocol to determine their effects on the treatments. The combinations of these values resulted in a choice of eight different treatment settings to be tested.

2.3.3 Preliminary study; Explanation of cracking of the laser treated surface

2.3.3.1 Introduction

In previous SEM studies of lased samples, cementum specimens showed several cracks. Some studies on untreated cementum suggested that cracks were produced by the coater machine with the vacuum causing the water content of the tissue to evaporate very quickly resulting in shrinkage and surface crack formation. This study was conducted to determine whether cracks on treated surface were due to the processing techniques or the impact of the laser pulses.

2.3.3.2 Material and methods

A silicon impression (Xantopren, Bayer, Germany) was obtained from one calculus specimen. After laser exposure

Fig.2.16 Silicon impression from the experimental surface (left); epoxy resin impression (right). (See the text).

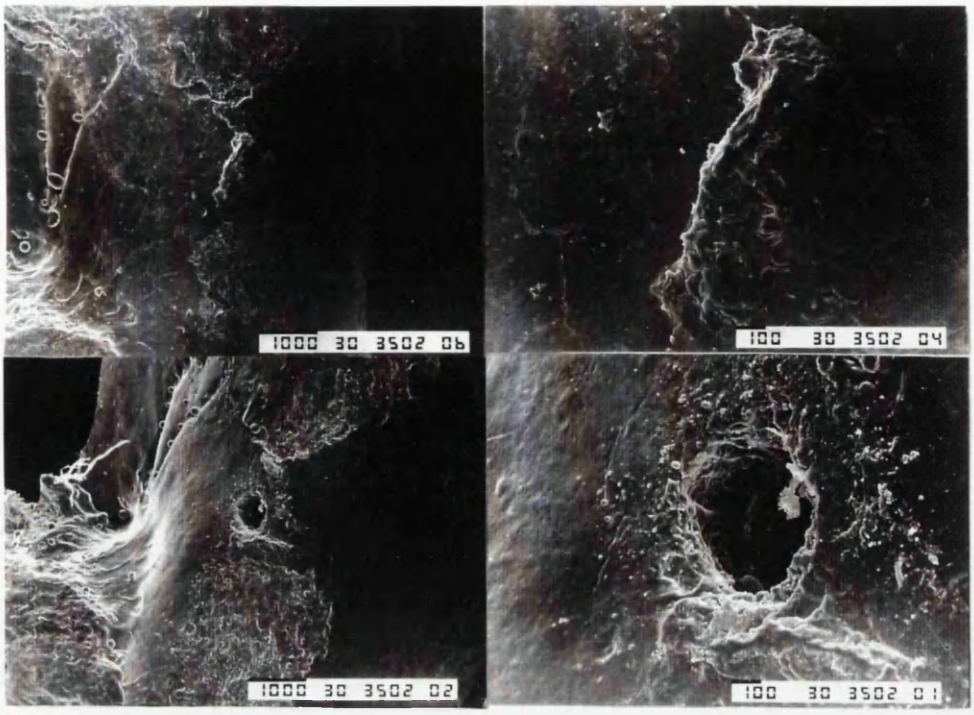

Fig.2.17 SEM micrographs of the replicated untreated (top left and top right), and the laser treated calculus surface (bottom left and bottom right) (preliminary study). No difference in the surface cracking is observed.

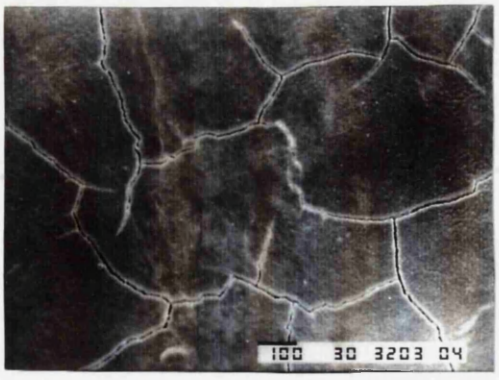

Fig.2.18 SEM micrograph from the untreated cementum surface. Cracking of the specimen was due to the specimen preparation process.

at 100 mJ, 20 pps for 10 sec, another silicon impression was taken. Both impressions were poured by epoxy resin (Epofix, Struers, England) to obtain replica models of tooth before and after laser treatment (Fig. 2.16).

In addition, 2 unlased cementum specimens were fixed and dried (as described in the sections 2.3.4.7 and 2.3.4.8). The replicas and the untreated cementum specimens were mounted on aluminium stubs and splutter-coated with gold and examined by SEM (sections 2.3.4.9 and 2.3.4.10).

2.3.3.3 Results and conclusion

Replicas reproduced the surface details accurately. SEM showed no difference in cracking of the two replicas taken before and after lasing (Fig. 2.17). The unlased cementum specimens showed cracks, similar to previous laser treated specimens (Fig. 2.18). Therefore, it was unlikely that the laser treatments had a major contribution to the surface cracking.

2.3.4 Material and methods used in the main *in vitro* laser experiment

2.3.4.1 Introduction

The first pilot study (section 2.3.1) demonstrated that the study of the effects of laser on the root surface was difficult if the laser handpiece was moved on the surface

during the treatment. The present study describes the effect of different laser settings and treatment durations on the calculus, cementum and dentine when a stationary handpiece is used to deliver the laser energy. The aims of this study were: 1) To determine whether there was a laser energy dose which could selectively ablate calculus without affecting cementum and dentine; 2) To determine whether the ablative effect of a given laser energy dosage on the calculus and root surface, is repeatable i.e. whether it could be used in a predictable manner. This study consisted of 2 separate experiment; one on subgingival calculus and the other on cementum and dentine. The results of these experiments have been described in chapter 3.

2.3.4.2 Tooth collection and preparation

Freshly extracted teeth were collected from the Oral Surgery Department of Glasgow Dental Hospital.

Calculus experiment: For the calculus study, each tooth had at least one flat surface with subgingival calculus as these are more easily assessed by SEM. Thirty-two teeth fulfilling the above criteria were selected.

Cementum and dentine experiment: For the experiment on the cementum and dentine, the criteria was to have a flat surface with no periodontal disease i.e. the root surface should have been exposed only after extraction. Six teeth were selected and the remnants of the periodontal ligament

were removed using forceps. This was confirmed by examining the roots at x10 magnification under a stereomicroscope. Of these 6 teeth, 3 were randomly selected and excessively root planed using a periodontal curette until it was judged that the cementum layer was removed, and the dentine became exposed. Using a sharp scraper, the root surfaces were divided into 8 areas, each approximately 2x2 mm. Debris from the scraping and root planing were removed gently using a soft brush and deionised water. Each area was subjected to one of the 8 laser treatments as described subsequently. All the teeth were kept in a 0.12% thymol solution in a refrigerator until the experiment.

2.3.4.3 Study design

Calculus experiment: Thirty two teeth were randomly divided into 8 groups of 4. Each group was then treated by one of the 8 laser protocols as described subsequently. Each tooth was treated on 2, 3 or 4 sites depending on the size and extent of calculus.

Cementum and dentine experiment: Three cementum covered and three root planed teeth were treated. Each of the 8 small areas described previously was randomly allocated to one of the laser treatments.

2.3.4.4 The Nd:YAG laser

The laser used in this study was an Nd:YAG laser, especially designed for dental practice (American Dental Laser, model d.lase-300, Sunrise Technology Inc., Fremont, California) (Fig. 2.19). The Nd:YAG laser beam has a wavelength of 1064 nm. Since the wavelength is not in the visible part of the spectrum, a visible and low energy He-Ne laser is used in the instrument as an aiming beam. The medium for the laser energy is a crystal rod made of yttrium, aluminium and garnet which has been seeded with neodymium. A timer is supplied into the energy source and gives the laser output a pulse mode, each pulse lasting for 150 mμs. The pulse rate ranges from 10 to 30 pulses per second (pps) which is adjustable by the operator. The energy ranges from 30 to 150 mJ per pulse. By varying the pulse rate and the pulse energy output, a range of average power settings from 0.3W to 3W can be achieved. The laser energy is transmitted through a flexible optic fibre cable which has an inner diameter of 320 μm. The energy is transferred to the target area by an adjustable handpiece fitted to the end of the cable. The laser is controlled by the operator using a foot pedal.

2.3.4.5 Laser usage and safety

Since the high energy Nd:YAG laser pulses are readily transmitted through the transparent eye tissues, the laser operator, assistants and patient wore special protective spectacles.

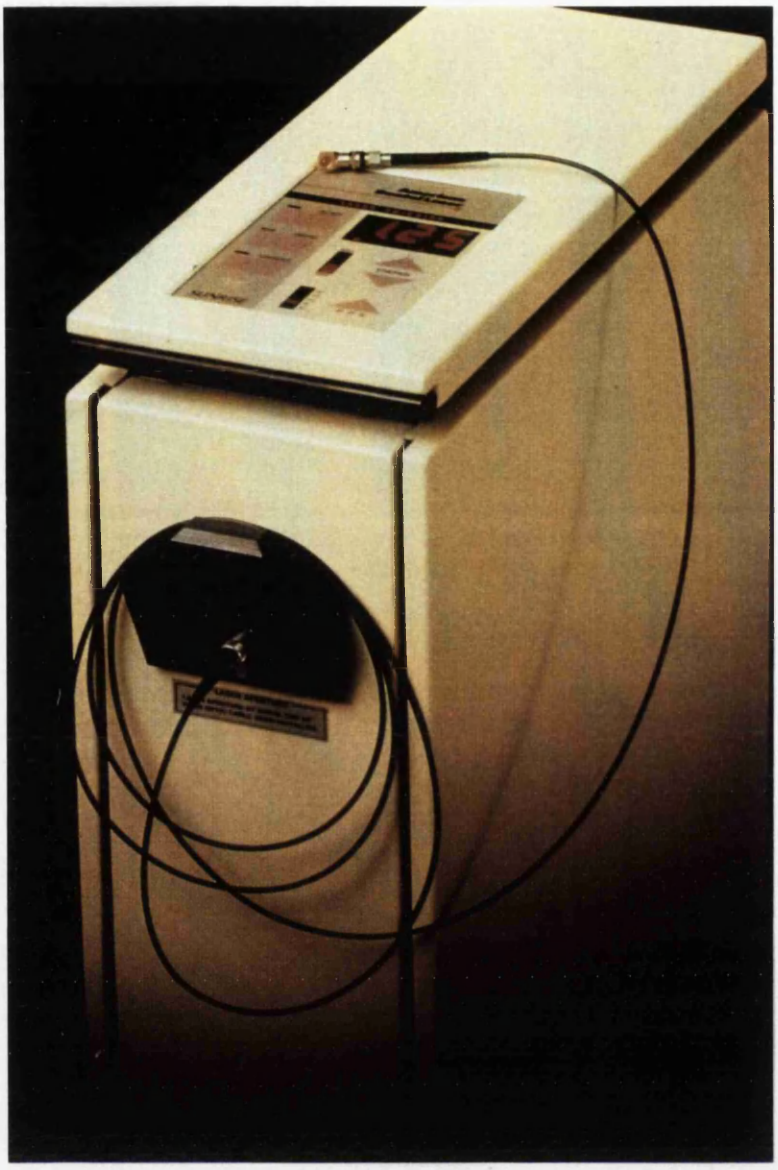

Fig.2.19 American Dental laser (Nd:YAG laser)

During application of laser energy to dental tissues, tip of the optical fibre may become damaged. When this happens, the fibre does not emit a uniform beam. Thus the beam emitted by the fibre was checked frequently by examining the integrity of the image of the aiming beam on a flat surface (which should be a full circle). If the fibre tip was damaged, its first few millimetres were cut using a special cutter and the handpiece was re-adjusted.

2.3.4.6 Laser treatment

The laser treatment settings were chosen from the results of a pilot study (section 2.3.2). These were:

Laser energy: 50 and 100mJ.
Number of pulses per second: 10 and 20pps.
Treatment duration: 1 and 5 sec.

Therefore, 8 laser treatment settings were used in the study.

Using an adjustable jig the handpiece tip was held stationary, in contact with, and perpendicular to the surface to be treated. The use of a stationary handpiece provides a standardised and repeatable method of applying the laser beam to the tissue. If the handpiece is moved over the entire surface, some areas might be treated more than others and some areas might be left untreated,

because of the small diameter of the laser beam, which is only 320 μm across.

2.3.4.7 Fixation

Immediately after treatment, the specimens were fixed in ice-cold 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.4 for a minimum of 2 hours.

2.3.4.8 Dehydration

After fixation the specimens were washed three times in 0.1M sodium cacodylate buffer, for 20 minutes each time, then dehydrated in increasing concentrations of ethyl alcohol as follows: 33% for 4 hours, 50% for 4 hours, 67% for 12 hours, 95% for 48 hours and 100% for 72 hours. The final dehydration was carried out by immersing the specimens in hexamethyldisilazane for 36 hours. The specimens were removed from this solution and air dried in a dust-free environment for 24 hours. Long dehydration times were required because of the large size of the specimens i.e. whole teeth, and also because of the dense, highly mineralised nature of the tissue.

The specimens were mounted on 32 mm diameter aluminium stubs (Agar Scientific Ltd.) using a conductive carbon cement (Leit C, TAAB Laboratories Equipment Ltd.).

2.3.4.9 Splutter coating

The specimen was placed in the vacuum chamber of a splutter coating machine (Polaron E 5000) and the air was evacuated until a reading of 0.1 Torr was obtained. At this time argon was allowed to bleed slowly into the chamber while it was still being pumped down. Argon is electrochemically neutral and is less likely to interfere with the gold coating process than air. A thin film of gold was evaporated from a target situated at the top of the chamber onto the surface of the specimen at a voltage of 1.2 kV. This process was allowed to continue for 5 minutes.

2.3.4.10 Scanning electron microscopy (SEM)

In this study, a Jeol T 300 scanning electron microscope operating at an accelerating voltage of 30KV and angulation of zero degrees was used. Micrographs were taken from the laser treated sites at x100 and x750 magnifications using Kodak TP120 film type 6415 (Kodak Ltd. Manchester).

2.3.4.11 Index of laser damage

The evaluation of the surface damage to the tissues produced by the laser was carried out as a blind examination by the investigator. The following criteria were used as an index to quantify the damage produced by the laser:

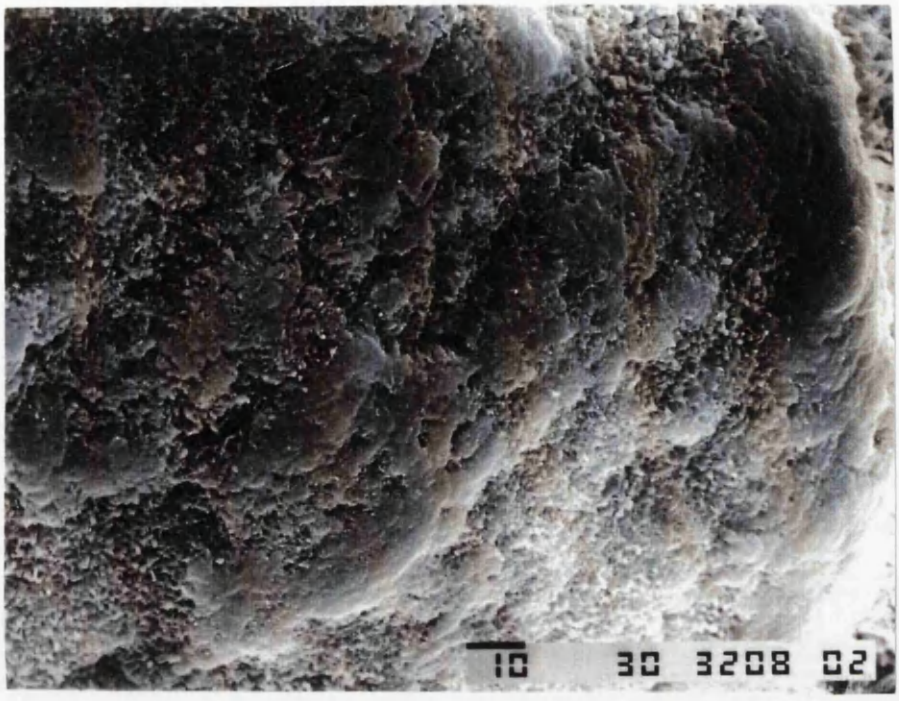

Fig. 2.20 Index for laser damage; Score 0 (see the text); magnification = x750.

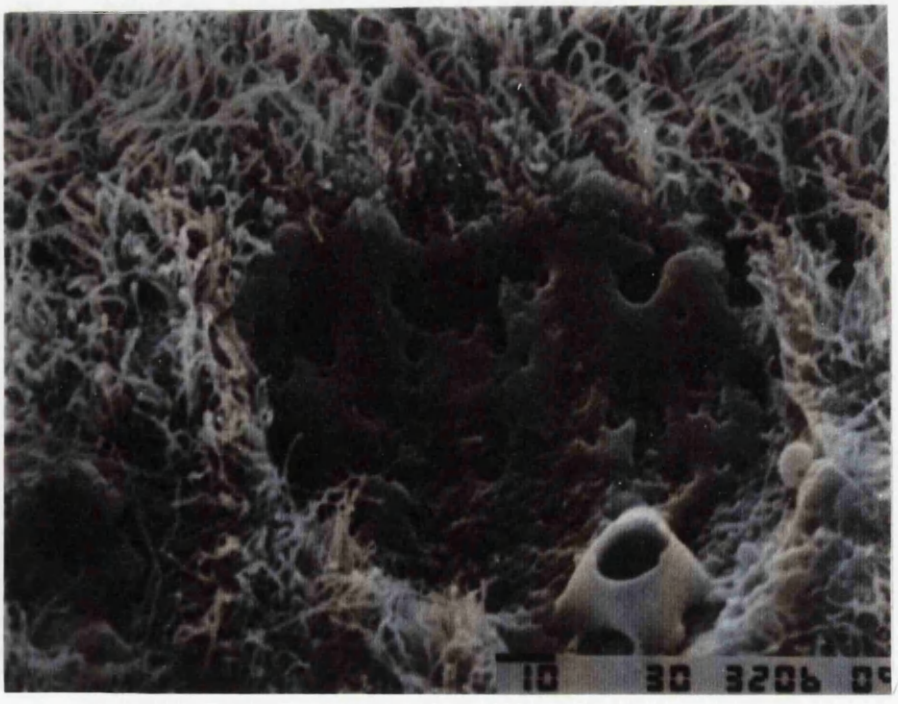

Fig. 2.21 Index for laser damage; Score 1 (see the text); magnification = x750.

Fig. 2.22 Index for laser damage; Score 2 (see the text); magnification = x100.

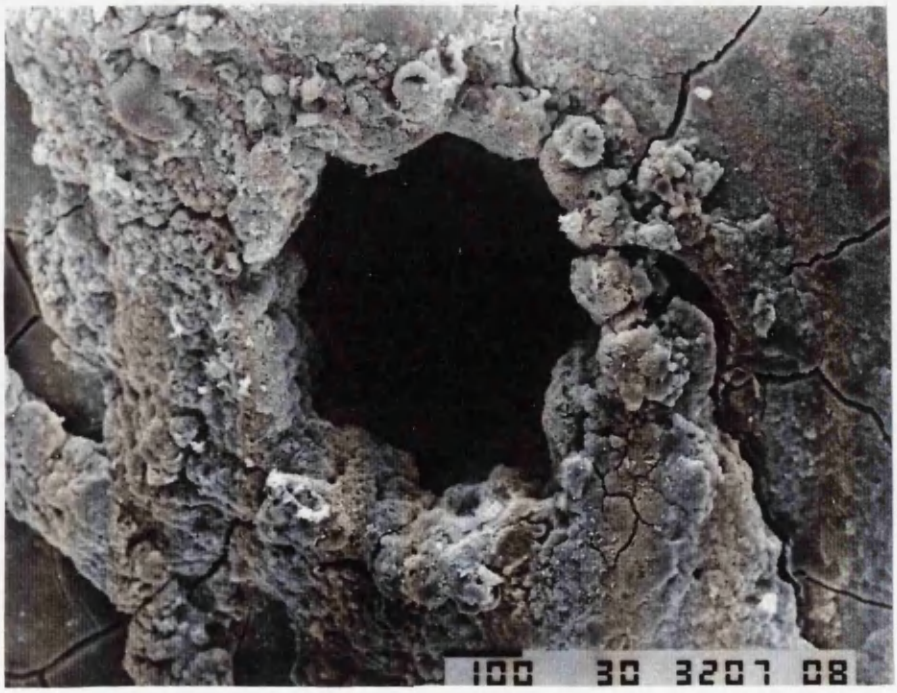

Fig. 2.23 Index for laser damage; Score 3 (see the text); magnification = x100.

Score 0: No change (Fig. 2.20).

Score 1: Slight changes including superficial melting of the surface. There is little or no ablated material. These changes are not visible at a magnification of x100 (Fig. 2.21) .

Score 2: Moderate changes, i.e. shallow depressions on the surface of the tissue caused by the laser. These changes are visible at a magnification of X100 (Fig. 2.22).

Score 3: Severe changes including deep craters caused by ablation and dissociation of surface material. These were visible on the tooth surface (Fig. 2.23).

2.3.4.12 Statistical analysis

The results from the experiment on cementum and dentine indicated that there was large variability between teeth in their response to the laser. Thus, in the calculus experiment, the tooth rather than the site was used as the unit of analysis and the index of damage was averaged over sites within a tooth to produce a single measure of damage for each tooth. The effect on tooth damage of energy setting (50 or 100mJ), the number of pulses per second (10 or 20), and the time (1 or 5 sec) was assessed by a three-way analysis of variance. The total energy input for each setting was evaluated by multiplying the energy (mJ), the number of pulses per second and time. The relationship between the logarithm of the total energy input and damage was assessed by linear regression.

2.4 The clinical laser study

2.4.1 Introduction; the rationale for the choice of laser settings

The literature on the laser studies were reviewed in details in chapter 1. However, it seems necessary here, to highlight the important points of the literature which helped determine the laser settings used in this clinical study:

Some investigators have suggested that lasers can reduce the bacterial population in the pocket and contaminated dentine (White *et al.*, 1991; Tseng *et al.*, 1991b; Cobb *et al.*, 1992). The main strategy in periodontal therapy is the elimination of bacterial plaque from periodontal pockets and root surfaces. Laser radiation of various wavelengths including Nd:YAG laser has been shown to be capable of killing bacteria to various extents (Cobb *et al.*, 1992; Stabholz *et al.*, 1993b; Sarkar and Wilson, 1993) and so in theory, could be used to substantially reduce the root surface bacterial population. The Nd:YAG laser, however, has been shown to alter the physical and biological properties of the cementum surface, if used at energies in the range commonly used (Cobb *et al.*, 1992; Spencer *et al.*, 1992; Trylovich *et al.*, 1992; Morlock *et al.*, 1992; Thomas *et al.*, 1992). Root surface damage has been reported to be caused by energy level of 80mJ at 10pps *in vitro* using a moving fibre (Trylovich *et al.*,

1992). Cobb *et al.* (1992) used energy settings of 1.75 W (about 87mJ, 20 pps) and higher *in vivo*, and reported a significant reduction in the proportion of periodontopathic bacteria. However, the cementum surface was damaged by the high levels of laser energy as demonstrated by scanning electron microscopy (SEM). Moreover, temperature rise beyond the thresholds tolerable for the pulp has been observed if the root surface was treated with laser energy settings higher than 80mJ, for a dentine thickness of 1mm (White *et al.*, 1994). It has also been reported that the Nd:YAG laser is capable of 90% kills or more for some oral bacteria including several periodontopathic species at an energy setting of 80mJ and 10 pulses per second (pps) for 3 minutes *in vitro* where the fibre was manipulated within 50µl microbial suspensions (Whitters *et al.*, 1994). On conclusion, it appeared that the 80mJ at 10pps, though low in energy level, had some bactericidal effect and thus warranted further investigation. Therefore, 80mJ at 10pps was included in the protocol. On the other hand, since some reports indicated that the laser-induced alterations may happen even at energies as low as 80mJ at 10pps (Trylovich *et al.*, 1992), 50mJ at 10pps was also included as a laser setting in the study for assessing its efficacy and also damage potentials on the root surface.

2.4.2 Study design; patient and site selection

Eleven patients with untreated chronic adult periodontitis who had periodontally affected teeth of poor prognosis and scheduled for extraction, took part in this study. In total 80 sites were selected, each receiving one of the four treatments described subsequently. In each patient, four pockets were matched for pocket depth within a tolerance of 1 mm. Nine patients provided 8 sites, that is two sites for each treatment modality, and two patients provided one site for each treatment. An informed consent form was signed by each patient.

Clinical measurements were performed for all 4 sites at baseline, and 6 weeks after treatment. Microbiological samples were taken at baseline, after treatment, and 6 weeks after treatment. After 6 weeks, test teeth were extracted as scheduled. Treated surfaces were examined using SEM for surface alterations.

2.4.3 Treatments

Treatments included lasing with 50mJ, or 80mJ pulses, or scaling; one site was the untreated control.

Pulsed Nd:YAG laser treatments were performed with an Nd:YAG laser with a contact fibre optic handpiece, at power settings of either 50mJ or 80mJ both at 10pps. The optical fibre was kept almost parallel to the root surface with the fibre tip in contact with the root surface during

treatment. The handpiece was moved back and forth by the operator to cover the whole surface area of each pocket for 3 minutes. Scaling and root planing were performed with periodontal hoes and curettes until a smooth surface was obtained (on average ten overlapping strokes). Laser treatments and scaling were preceded by local anaesthetic administration. Control treatment included applying the laser handpiece to the root surface of control sites for 3 minutes with the laser machine switched off, taking into account any effect of the fibre in mechanically removing bacteria.

2.4.4 Clinical measurements

Baseline measurements included, Plaque Index, Modified Gingival Index, subgingival microbiologic sampling, pocket depth and presence of bleeding on probing. Immediately after treatment microbiologic sampling was repeated. The clinical and microbiological measurements were repeated after 6 weeks.

2.4.5 The Plaque Index and the Modified Gingival Index

These indices were described in the sections 2.2.5.4, and 2.2.5.5.

2.4.6 Pocket depth

Using a pressure sensitive probe with a standard pressure of 25 g, selected sites were measured to the nearest millimetre. The probe was inserted immediately

buccally/lingually at the contact point, being parallel to the long axis of the tooth.

2.4.7 Bleeding index

Dichotomous values of bleeding index were obtained. At each site, the presence or absence of bleeding up to 30 seconds after gentle probing at each site was inspected and recorded at the same time as the pocket depth measurement.

2.4.8 Microbial sampling and culture

Microbial samples were taken from each site before, immediately following, and 6 weeks after treatment. Samples were collected by first removing supragingival plaque, then isolating the site with cotton rolls. A sterile, medium-size paper point was inserted to the depth of the pocket for 10 seconds and transferred into a bottle containing Fastidious Anaerobe Broth (FAB) (Lab M. England) and immediately transferred to the laboratory for total anaerobic count (CFU/ml). Each sample was Vortex mixed for 30 sec, serially diluted in FAB from neat to 10^{-5} and inoculated with a spiral plater (Don Whitley Scientific, Shipley, England) onto Fastidious Anaerobe Blood agar containing 7.5% v/v sterile defibrinated horse blood. Plates were incubated for 10 days at 37° C in an anaerobic incubator (Don Whitley Scientific, Shipley, England) at an atmosphere of 5% H_2, 10% CO_2, 85% Nitrogen. The total number of colony forming units in each sample

(CFU/ml) was calculated and converted to logs for statistical analysis.

2.4.9 Extraction and SEM

After the 6-week visit, the laser treated teeth were extracted and were then fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.4. Following fixation, the specimens were dehydrated using an ascending series of graded ethanol solutions and the final dehydration was carried out using hexamethyldisilazane for 36 hours. The specimens were splutter-coated (Polaron E5000) with gold and viewed on a Jeol JSM T300 SEM at 30 KV to examine whether the laser damage was present on the root surfaces. The characteristic features of the laser damaged surface include charring, crater formation, cementum melt down, with subsequent resolidification of inorganic material (Cobb *et al.*, 1992; Trylovich *et al.*, 1992).

2.4.10 statistical analysis

For the analysis of plaque and gingival index data, baseline and 6-week visit data were compared using Wilcoxon's test and changes in these parameters in the 4 groups were subjected to Friedman's test. The latter compares the mean ranks of treatments i.e. an average of every site's score, ranked across the four treatment groups within each patient. BOP data of baseline and 6-week visit in each treatment group were compared using the McNemar's test. Pocket depths were compared using a

paired t-test on baseline and 6-week visit data for each treatment group. Repeated measurement analysis of variance (ANOVA) was used to compare pocket depth changes in 4 groups, and where an overall significant difference was detected, Duncan's multiple range test was used to uncover the significantly different groups. For the microbiology data, differences between the counts in samples before and immediately after treatment, as well as differences between the counts before treatment and at the 6-week visit, were subjected to repeated measures ANOVA and Duncan's multiple range test.

CHAPTER III

RESULTS

3.1 The clinical study of local antimicrobial delivery systems

3.1.1 Introduction

This study was carried out on maintenance patients who, despite previous mechanical therapy, still had sites with signs of periodontal disease. The study aimed to investigate the clinical effects on the periodontitis sites of 3 commercially available locally delivered antimicrobial systems as adjuncts to scaling and root planing. This section contains the results of the study from baseline to the 6 month follow-up. Firstly, subject information such as number of participants, subjects dropping out, and the demographic characteristics are presented. The baseline clinical data will be presented in the next section. Then, the probing pocket depth data throughout the study is presented together with the analysis of the effect of baseline probing depth on treatment outcome. This is then followed by the attachment level changes and changes in recession. The next sections deal with bleeding on probing, suppuration, gingival scores, plaque scores, the effect of smoking on treatment outcome and GCF volume data. Finally, the adverse effects and the time taken for each treatment are presented.

3.1.2 Dropped out subjects and excluded sites

A total of 67 patients fulfilled the inclusion criteria and agreed to take part in the study. Four suitable sites

(as described in section 2.2.5.1) were selected within each patient, impressions were taken and acrylic occlusal stents were fabricated. However, out of 67 subjects, 9 subjects did not attend for the baseline measurements, one patient failed to complete the treatment course on time and therefore was excluded, another patient completed the treatment but did not attend at the 6 week visit and was excluded from the study and finally, two other patients discontinued with the study before the 6 month measurements, and therefore, the data corresponding to them were excluded from the analysis.

Fifty four patients completed the study with the scaling alone and the scaling plus tetracycline fibre groups having 13 patients each and the scaling plus minocycline gel and the scaling plus metronidazole gel groups having 14 patients each. One patient who received scaling plus tetracycline fibre, had one site which had to be excluded from the analysis because of accidental early removal of the fibre from the pocket. One site in the scaling plus metronidazole gel group and one site in the scaling alone group were excluded because these sites had been missed during examination and the wrong sites measured during one of the visits. Therefore, in the scaling plus minocycline gel group 56 sites were treated and completely followed up, whereas in the scaling plus metronidazole gel group this was 55 sites and in the remaining 2 groups 51 sites

with complete data set were available, resulting in a total of 213 sites in the four treatment categories.

3.1.3 Study visits

Patients included in the study were seen in 379 visits during the course of the study. Fifty four visits were required for the screening, site selection and impression taking; 216 visits were required for the measurements and finally 109 visits were required for the treatments including scaling and root planing under local anaesthesia and application of antimicrobial systems, re-application of the gels, and removal of the fibres. In addition to the 109 visits required for the treatment of the selected sites, some additional visits were required for the routine maintenance care of the other sites.

3.1.4 Demographic characteristics of the patients

Demographic characteristics of the 54 patients who completed the study are shown in Table 3.1. Of the 54 patients, 52 were white Caucasians and 2 were Asians. The subject population included 22 male (40.7%) and 32 female (59.3%). The average age of participants in the study was 44.6 (ranging from 30 to 67). The average age of the subjects in the 4 groups were rather close and no significant difference existed among them (p=0.370). Twenty-eight (51.9%) of the subjects were smoker. The distribution of smokers in the 4 treatment groups were

ble 3.1 Demographic characteristics of the 54 subjects 4 treatment groups.

	Tetracycline fibre (n=13)	Minocycline gel (n=14)	Metronidazole gel (n=14)	Scaling alone (n=13)
Age ear)	47.85 ± 1.91	43.57 ± 1.66	44.21 ± 3.11	42.69 ± 1.38
moker (%)	8 (61.5%)	8 (57.1%)	6 (42.9%)	6 (46.2%)
le (%)	6 (46.2%)	6 (42.9%)	3 (21.4%)	7 (53.8%)
thnic tegory	13 Caucasians	14 Caucasians	13 Caucasians & 1 Asian	12 Caucasians & 1 Asian

ble 3.2 Baseline clinical data (mean ± standard error of mean) among the 4 treatment groups (n = 54 subjects).

	Tetracycline fibre (n=13)	Minocycline gel (n=14)	Metronidazole gel (n=14)	Scaling alone (n=13)
ocket epth(mm)	5.512 ± 0.173	5.563 ± 0.186	5.597 ± 0.185	5.471 ± 0.226
BOP	0.916 ± 0.037	0.911 ± 0.050	0.845 ± 0.058	0.961 ± 0.026
Pus	0.115 ± 0.046	0.071 ± 0.031	0.149 ± 0.052	0.160 ± 0.061
MGI	2.288 ± 0.113	1.911 ± 0.188	1.988 ± 0.111	2.282 ± 0.103
PI	0.500 ± 0.133	0.607 ± 0.163	0.613 ± 0.112	0.685 ± 0.103

rather similar (p=0.35, chi-square=1.268, chi-square test).

3.1.5 Baseline data

Table 3.2 shows the clinical data obtained at the baseline visits among the 4 patient groups. There was no significant difference between the mean probing pocket depth among treatment groups at the baseline (p=0.970, one-way ANOVA). The baseline probing depth (patient derived data) ranged from 4.375 mm to 7.25 mm. The overall mean probing depth was 5.54 mm with the standard error of the mean of 0.09. Although the selected sites had to be at least 5 mm in probing depth to be selected at the screening visit, when the 'Florida Probe' was used instead of manual probe at the baseline visits, 84 sites showed probing depth less than 5 mm, and 4 sites showed probing depth less than 4 mm. All the selected sites had bleeding on probing or suppuration using manual probe at the screening visit, whereas at the baseline visit, when the 'Florida Probe' was used 200 sites were positive for either bleeding on probing or suppuration and 13 sites showed neither bleeding on probing nor suppuration. There was no significant difference between treatment groups with regard to bleeding on probing at baseline (p= 0.891). The Plaque Index baseline scores were relatively low among the treatment groups with an average of 0.60 ± 0.06 indicating a general good oral hygiene level at the baseline. There was no significant difference in the

plaque scores between treatments at the baseline (p=0.570). The Modified Gingival Index (MGI) data had an average of 2.11 and a standard error of the mean of 0.07 at the baseline. No significant difference existed in the baseline MGI data between treatment groups (p= 0.122).

3.1.6 Mean reduction in probing pocket depth

Site-based measurements were pooled for each patient at each visit to produce a single figure of probing pocket depth or attachment level. By doing so, each figure represented an 'independent observation'. This would not have held true, if a site-based analysis had been performed. This particularly seemed important in the present study in which antimicrobial drugs were utilised and probably a considerable carry-across effect occurred between the sites. In addition, since almost all patients provided an equal number of sites, each site equally contributed to the overall data even after averaging the site data within patients.

Table 3.3 shows the probing pocket depth data derived from the patients as the unit of analysis. At the 6 week visit all 4 treatments resulted in a highly significant reduction in the probing pocket depth compared to baseline (p<0.001). This significant difference was sustained in all groups until the end of study period. Table 3.4 shows the reduction of probing pocket depth among the 4 treatment groups at different follow-up visits. The data

le 3.3 Probing depth data (derived from 'patient' as unit of analysis) at different visits. Mean ± Standard or of the mean and 95% confidence intervals are shown. obability values are obtained by paired t-tests between eline and follow-up visits. The unit of measurements is mm.

Time point	Baseline	6 week	3 month	6 month
racycline fibre (n=13)	5.51 ± 0.17 (5.14 , 5.89)	4.16 ± 0.13 (3.88 , 4.45) **p<0.001**	4.06 ± 0.14 (3.75 , 4.36) **p<0.001**	4.13 ± 0.18 (3.73 , 4.53) **p<0.001**
ocycline gel (n=14)	5.56 ± 0.19 (5.16 , 5.97)	4.81 ± 0.23 (4.21 , 5.18) **p<0.001**	4.63 ± 0.24 (4.13 , 5.17) **p<0.001**	4.74 ± 0.20 (4.32 , 5.14) **p<0.001**
ronidazole gel (n=14)	5.60 ± 0.19 (5.20 , 6.00)	4.65 ± 0.20 (4.21 , 5.09) **p<0.001**	4.49 ± 0.26 (3.93 , 5.05) **p<0.001**	4.58 ± 0.23 (4.09 , 5.07) **p<0.001**
.ing alone (n=13)	5.47 ± 0.23 (4.98, 5.96)	4.87 ± 0.26 (4.29, 5.44) **p=0.001**	4.58 ± 0.32 (3.88, 5.27) **p<0.001**	4.73 ± 0.33 (4.01, 5.44) **p=0.017**

indicated that the major pocket depth reduction took place during the first 6 weeks post-treatment. The healing response as defined by pocket depth reduction maximised at month 3 for all treatments. At the 6 month visit, there was a slight relapse towards the initial values in all groups. When the baseline pocket depth was taken into account as a covariate, the adjusted mean pocket depth reductions only slightly varied from the original values (Table 3.4).

At the 6 week visit, the pocket depth reduction was the greatest in the scaling plus tetracycline fibre group with 1.35 mm followed by the scaling plus metronidazole gel group and the scaling plus minocycline gel group with 0.95 mm and 0.87 mm respectively. At this time point, the lowest pocket depth reduction was observed in the scaling alone group with a value of 0.60 mm (Fig. 3.1).

Table 3.5 shows an analysis of variance on the 6 week probing depth changes using the 'General Linear Model' procedure with the baseline pocket depth as a continuous covariate and the treatment as the main effect. Initial analysis (Table 3.5a) disclosed that there was no interaction between the covariate and the main effect and covariate slopes were not heterogeneous across the treatment groups. In other words, the effect of baseline pocket depth on the magnitude of the change in probing depth was fairly similar among the groups. Therefore, the

Table 3.4 Pocket depth reduction from baseline among the 4 groups at different follow-up visits. The values in parenthesis are covariate adjusted pocket depth reductions using baseline pocket depths as a covariate. The values in the second row of each cell are 95% confidence intervals for unadjusted reductions.

Treatment	6 week	3 month	6 month
Tetracycline fibre (n=13)	1.35 (1.36) 0.97 , 1.73	1.45 (1.46) 1.03 , 1.88	1.38 (1.39) 0.89 , 1.87
Minocycline gel (n=14)	0.87 (0.86) 0.58 , 1.16	0.91 (0.91) 0.54 , 1.29	0.82 (0.81) 0.58 , 1.07
Metronidazole gel (n=14)	0.95 (0.93) 0.60 , 1.30	1.11 (1.09) 0.57 , 1.65	1.02 (1.00) 0.53 , 1.51
Scaling alone (n=13)	0.60 (0.62) 0.30 , 0.91	0.89 (0.91) 0.46 , 1.33	0.74 (0.77) 0.16 , 1.33

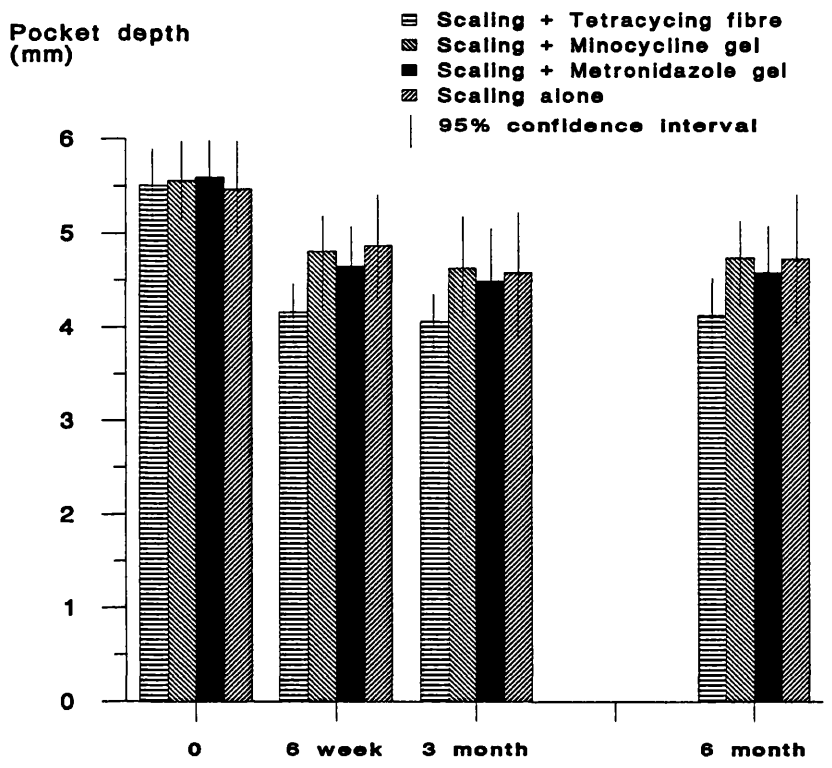

Fig.3.1 Probing pocket depth among the treatment groups at the baseline, 6-week, 3-month and 6-month visits. Means and 95% confidence intervals are displayed.

Table 3.5 Analysis of variance for pocket depth changes at 6 week considering the baseline pocket depth as a covariate (PD0: baseline pocket depth, Treat: treatment)

a) Including the interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	3	3.7346	1.1954	0.3985	1.42	0.248
PD0	1	1.1326	1.6269	1.6269	5.81	0.020
TREAT*PD0	3	1.7339	1.7339	0.5780	2.06	0.118
Error	46	12.8890	12.8890	0.2802		
Total	53	19.4901				

b)After removing the insignificant interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
PD0	1	1.1845	1.1326	1.1326	3.80	**0.057**
TREAT	3	3.6828	3.6828	1.2276	4.11	**0.011**
Error	49	14.6228	14.6228	0.2984		
Total	53	19.4901				

c) Post-hoc comparisons*:

	Tetracycline fibre	**Minocycline gel**	**Metronidazole gel**
Minocycline gel	p=0.022		
Metronidazole gel	p=0.045	p=0.726	
Scaling alone	p=0.002	p=0.268	p=0.146

* After the Bonferroni correction for multiple comparisons, the threshold for statistical significance was $p<0.020$

model was tested again without this interaction term (Table 3.5b). This analysis disclosed the presence of a significant difference among the treatment groups (p=0.011). The post-hoc comparisons were performed by comparing only 2 treatment groups at a time (Table 3.5c). After adjustment of the probability values for multiple comparisons, the only significantly different pair was found to be the scaling plus tetracycline fibre and the scaling alone groups (p=0.002).

At the 3 month visit, the same rank as the 6 week visit existed between the treatments with regard to the pocket depth reductions. However, the mean reduction in the scaling alone group was quite close to the scaling plus minocycline gel group. In fact, the covariate adjusted mean reductions were exactly the same (0.91 mm). The mean pocket depth reductions were 1.45 mm, 1.11 mm, 0.91 mm and 0.89 mm for the scaling plus tetracycline fibre, scaling plus metronidazole gel, scaling plus minocycline gel and scaling alone groups respectively. At the 6 month visit the reductions in the pocket depth still had the same rank with the reductions as follow; 1.38 mm for the scaling plus tetracycline fibre group, 1.02 mm for the scaling plus the metronidazole gel group, 0.82 mm for the scaling plus minocycline gel group, and 0.74 mm for the scaling alone.

Table 3.6 Analysis of Variance for pocket depth changes at 3 month considering the baseline pocket depth as a covariate (PD0: baseline pocket depth, Treat: treatment)

a) Including the interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	3	2.6565	2.2040	0.7347	1.36	0.267
PD0	1	1.5319	2.2879	2.2879	4.23	0.045
TREAT*PD0	3	2.7982	2.7982	0.9327	1.73	0.175
Error	46	24.8641	24.8641	0.5405		
Total	53	31.8506				

b)After removing the insignificant interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
PD0	1	1.5260	1.5319	1.5319	2.71	**0.106**
TREAT	3	2.6623	2.6623	0.8874	1.57	**0.208**
Error	49	27.6623	27.6623	0.5645		
Total	53	31.8506				

Table 3.7 Analysis of Variance for pocket depth changes at 6 month considering the baseline pocket depth as a covariate (PD0: baseline pocket depth, Treat: treatment)

a) Including the interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	3	3.2003	1.1362	0.3787	0.67	0.577
PD0	1	3.1777	3.8311	3.8311	6.75	0.013
TREAT*PD0	3	1.6213	1.6213	0.5404	0.95	0.424
Error	46	26.1206	26.1206	0.5678		
Total	53	34.1200				

b)After removing the insignificant interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
PD0	1	3.1965	3.1777	3.1777	5.61	**0.022**
TREAT	3	3.1815	3.1815	1.0605	1.87	**0.146**
Error	49	27.7419	27.7419	0.5662		
Total	53	34.1200				

Tables 3.6 and 3.7 show the analysis of variance procedures for the 3 month and 6 month changes in the probing pocket depth. Similar to the 6 week data, no interaction was found between the baseline pocket depth and the treatments and the models had to be tested without the interaction terms. There was no significant difference between the treatments at the 3 month (p=0.208) and the 6 month visits (p=0.146).

3.1.7 Mean change in probing attachment level

Table 3.8 shows the mean change in the probing attachment levels among the 4 treatment groups during the 6 month follow-up after treatment. The same rank as observed in the probing depth reductions existed between the treatment groups with regard to the attachment level changes. By the 6 week visit, a significant attachment gain took place in all 4 treatment groups and lasted throughout the study period. However, at this time point, the change in the scaling alone group was smaller than the other 3 groups. In the 3 adjunctive antimicrobial treatment groups, the major attachment gain occurred during the first 6 weeks post-therapy and the 3 month attachment levels only slightly varied from the 6 week visit. However, in the scaling alone group the increase in the attachment level continued at about the same rate as the first 6 week post-treatment. At this time point, the attachment gains were 0.70 mm in the 2 groups of the scaling plus tetracycline fibre and scaling plus metronidazole gel (0.71 and 0.69

respectively for the covariate adjusted values), 0.50 mm for the scaling plus minocycline gel group, closely followed by the scaling alone group with 0.46 mm.

While in the scaling plus metronidazole gel group some relapse towards the baseline values was observed at the 6 month visit, the other 3 groups remained almost unchanged until the end of study. When the attachment level changes were adjusted for small differences in the baseline probing pocket depth among the treatment groups, only small variation existed between the original values and the covariate-adjusted values.

At the 6 month visit, the attachment gain in the tetracycline fibre group was still the greatest with 0.70 mm, followed by the other 3 groups which had relatively similar values (0.53, 0.51 and 0.45 mm for the metronidazole gel, minocycline gel and scaling alone groups respectively).

Tables 3.9 to 3.11 show the analysis of variance on the attachment level changes at the 6 week, 3 month and 6 month visits respectively. The General Linear Model procedures were used with the baseline probing pocket depth as covariate and the treatment as the main factor. No significant interaction was found between the treatment and the covariate, indicating that the enhancing effect of having deeper baseline pocket depth did not vary

Table 3.8 Attachment level changes from baseline among the 4 groups at different follow-up visits. The values in () are covariate-adjusted attachment level changes using baseline pocket depths as a covariate. The values in the second row of each cell are 95% confidence intervals for unadjusted reductions. The unit of measurements is mm.

Treatment	6 week	3 month	6 month
Tetracycline fibre (n=13)	0.75 (0.75) 0.49 , 1.00	0.70 (0.71) 0.37 , 1.04	0.70 (0.71) 0.28 , 1.12
Minocycline gel (n=14)	0.45 (0.44) 0.16 , 0.74	0.50 (0.49) 0.20 , 0.80	0.51 (0.49) 0.25 , 0.75
Metronidazole gel (n=14)	0.57 (0.56) 0.26 , 0.90	0.70 (0.69) 0.28 , 1.13	0.53 (0.53) 0.11 , 1.00
Scaling alone (n=13)	0.26 (0.28) 0.03 , 0.50	0.46 (0.47) 0.19 , 0.72	0.45 (0.46) 0.06 , 0.83

Table 3.9 Analysis of Variance for attachment level changes at 6 week considering the baseline pocket depth as a covariate (PD0: baseline pocket depth, Treat: treatment).

a) Including the interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	3	1.6372	0.1941	0.0647	0.31	0.816
PD0	1	1.4424	1.5931	1.5931	7.71	0.008
TREAT*PD0	3	0.2951	0.2951	0.0984	0.48	0.701
Error	46	9.5068	9.5068	0.2067		
Total	53	12.8814				

b)After removing the insignificant interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
PD0	1	1.5150	1.4424	1.4424	7.21	**0.010**
TREAT	3	1.5646	1.5646	0.5215	2.61	**0.062**
Error	49	9.8018	9.8018	0.2000		
Total	53	12.8814				

Table 3.10 Analysis of Variance for attachment level changes at 3 month considering the baseline pocket depth as a covariate (PD0: baseline pocket depth, Treat: treatment)

a) Including the interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	3	0.6937	0.7469	0.2490	0.78	0.511
PD0	1	1.0023	1.2119	1.2119	3.80	0.057
TREAT*PD0	3	0.9266	0.9266	0.3089	0.97	0.415
Error	46	14.6589	14.6589	0.3187		
Total	53	17.2815				

b)After removing the insignificant interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
PD0	1	1.0538	1.0023	1.0023	3.15	**0.082**
TREAT	3	0.6422	0.6422	0.2141	0.67	**0.573**
Error	49	15.5856	15.5856	0.3181		
Total	53	17.2815				

Table 3.11 Analysis of Variance for attachment level changes at 6 month considering the baseline pocket depth as a covariate (PD0: baseline pocket depth, Treat: treatment)

a) Including the interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	3	0.4707	0.3172	0.1057	0.27	0.843
PD0	1	2.8119	2.6779	2.6779	6.96	0.011
TREAT*PD0	3	0.2985	0.2985	0.0995	0.26	0.855
Error	46	17.6909	17.6909	0.3846		
Total	53	21.2720				

b)After removing the insignificant interaction term:

Source	DF	Seq SS	Adj SS	Adj MS	F	P
PD0	1	2.8173	2.8119	2.8119	7.66	0.008
TREAT	3	0.4653	0.4653	0.1551	0.42	0.738
Error	49	17.9894	17.9894	0.3671		
Total	53	21.2720				

significantly between groups. The null hypothesis testings were repeated without the interaction term in the model. The 6 week difference between the treatments did not reach the significance level (p= 0.062). The analysis of 3 month and 6 month changes also revealed no significant difference in the attachment level changes between groups (p= 0.573 and p=0.784 respectively).

3.1.8 The effect of baseline probing pocket depth on the treatment outcome

Since it was found that the baseline probing depth had a considerable and often significant effect on the treatment outcome i.e. pocket depth reduction and attachment gain (Tables 3.5 to 3.7 and 3.9 to 3.11), in order to further highlight this effect, all the sites were divided into 3 categories according to their initial probing depth values and regardless of their treatments. The categories were: a) pocket depth < 5 mm; b) 6.5 mm > pocket depth ≥ 5 mm; and c) pocket depth ≥ 6.5 mm. Table 3.12 displays the changes of pocket depth and attachment level among the 3 mentioned categories throughout the study period. This Table indicates that the sites with deeper initial pocket depth generally responded better to the treatment than the shallower sites.

3.1.9 Change in the recession

The method used in this study would not allow for measurements of initial recession in the study sites

Table 3.12 Changes in pocket depth and attachment levels at different follow-up visits. Sites were broken down by their initial pocket depth.

Baseline		Pocket depth reduction (mm)		Attachment gain (mm)	
	n	MEAN	SEM	Mean	SEM
		6 week visit:			
PD0<5	84	0.57	0.09	0.37	0.08
5≤PD0<6.5	91	1.01	0.09	0.46	0.07
PD0≥6.5	38	1.48	0.21	0.93	0.19
		3 month visit:			
PD0<5	84	0.75	0.10	0.41	0.09
5≤PD0<6.5	91	1.04	0.10	0.55	0.09
PD0≥6.5	38	1.90	0.26	1.08	0.20
		6 month visit:			
PD0<5	84	0.68	0.11	0.32	0.10
5≤PD0<6.5	91	0.92	0.12	0.45	0.09
PD0≥6.5	38	1.93	0.28	1.13	0.24

SEM: Standard error of the mean
PD0: baseline pocket depth

Table 3.13 Change in the recession (Mean ± standard error of the mean) among the 4 treatment groups at 6 week, 3 month and 6 month visits. The unit of measurements is mm.

Time point	6 week	3 month	6 month
Tetracycline fibre	0.60 ± 0.11	0.75 ± 0.13	0.68 ± 0.21
Minocycline gel	0.42 ± 0.10	0.42 ± 0.08	0.32 ± 0.10
Metronidazole gel	0.37 ± 0.14	0.41 ± 0.15	0.46 ± 0.13
Scaling alone	0.34 ± 0.15	0.44 ± 0.20	0.30 ± 0.16
One-way ANOVA	F=0.86 ***p*=0.470**	F=1.30 ***p*=0.286**	F=1.36 ***p*=0.266**

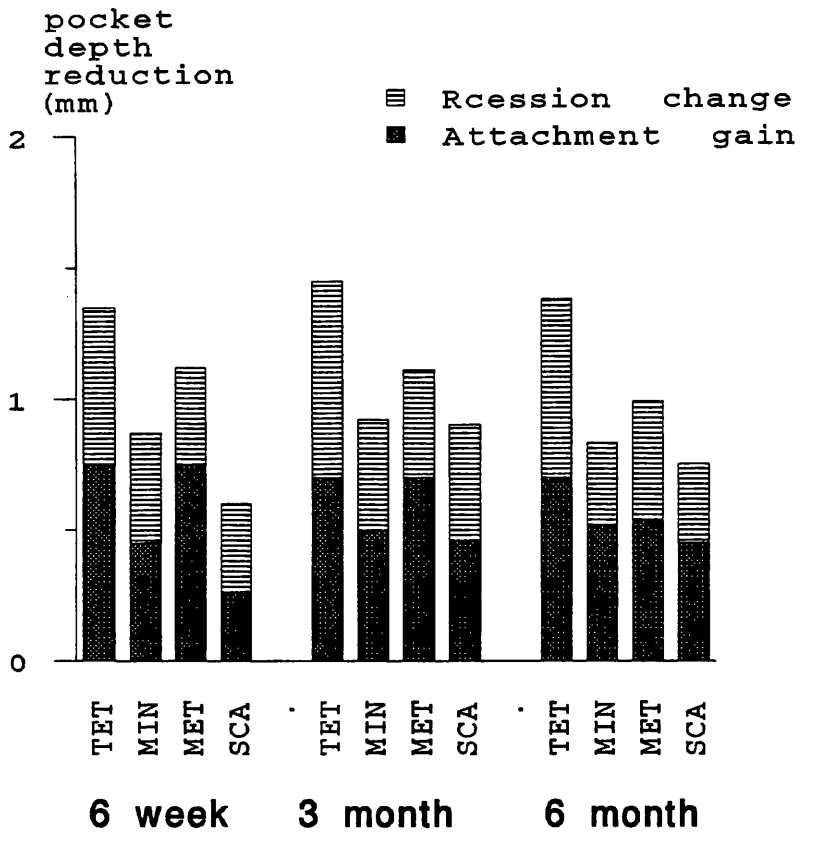

Fig. 3.2 Mean probing depth reduction, attachment gain, and recession changes among the treatment groups at 6 week, 3 month and 6 month visits (TET: scaling and root planing & tetracycline fibre; MIN: scaling and root planing & minocycline gel; MET: scaling and root planing & metronidazole gel; SCA: scaling and root planing alone).

because the cemento-enamel junction (CEJ) was not used as the reference point but the attachment levels were measured from an arbitrarily fixed point on the acrylic occlusal stent. The changes in the recession for each site was calculated by subtracting the attachment level changes from the pocket depth changes. Table 3.13 shows the changes in the recession among the treatment groups at the follow-up visits. For each follow-up visit, the site specific data within each patient was pooled to provide a single figure which represented the patient's change in the recession from the baseline until that time-point. All the treatments resulted in an increase in recession. By the end of the 6 month follow-up the greatest recession was observed in the scaling plus tetracycline fibre group followed by the scaling plus metronidazole gel, scaling plus minocycline gel and the scaling alone groups (Fig. 3.2). There were no significant differences in the recession changes between the groups at any time point (p=0.470, p=0.286, and p=0.266 for the 6 week, 3 month and the 6 month follow-up visits respectively).

3.1.10 Changes between visits

Between visit changes in the pocket depth and attachment levels were analysed by dividing the whole follow-up period of the study into 3 phases; the early, intermediate and late phases. The early changes were the 6 week changes and were presented in the sections 3.1.6 and 3.1.7. The intermediate changes were the changes which

occurred between the 6 week visit and the 3 month visit and were calculated by subtraction of the 6 week changes from the 3 month changes. The late changes were the changes which took place between the 3 month visit and the 6 month visit and were calculated by subtraction of the 3 month changes from the 6 month changes. Tables 3.14 and 3.15 display the mentioned changes for the pocket depth and attachment level respectively. At the early phase of the follow-up period, the reduction in the pocket depth and increase in the attachment level were the least in the scaling alone group. The intermediate changes in the 3 adjunctive antimicrobial groups were small and insignificant (p=0.45, p=0.60 and p=0.41 for the tetracycline fibre, minocycline gel and metronidazole gel groups respectively). However, in the scaling alone group, significant pocket depth reduction and attachment gain continued to occur during the intermediate phase of the follow-up (p=0.047 and p=0.038 for the pocket depth reduction and the attachment gain respectively). The changes in the late phase i.e. the interval between 3 and 6 month follow-up visits, were small. All groups showed some degree of relapse in the pocket depth with the greatest relapse in the scaling alone group. None of the pocket depth or attachment changes in the late phase of the follow-up period were significant.

Table 3.14 'Between-visit' changes for pocket depth; The early change was the change from baseline until week 6, the intermediate change was calculated by subtraction of the 3 month pocket depth from the 6 week pocket depth. The late change was calculated by subtraction of the 6 month pocket depth from the 3 month pocket depth. Mean ± standard error of the mean are shown. The p values in each cell represent paired t-test in changes occurred during the interval between the 2 consecutive visits. The data are subject derived (patient as the unit of analysis).

Between-visit change	Between baseline & week 6 (early change)	Between week 6 & month 3 (intermediate change)	Between month 3 & month 6 (late change)
Tetracycline fibre	1.35 ± 0.17 *p*<0.001	0.10 ± 0.13 *p*=0.45	-0.07 ± 0.20 *p*=0.72
Minocycline gel	0.87 ± 0.13 *p*<0.001	0.07 ± 0.09 *p*=0.60	-0.13 ± 0.16 *p*=0.55
Metronidazole gel	0.95 ± 0.16 *p*<0.001	0.13 ± 0.21 *p*=0.41	-0.6 ± 0.16 *p*=0.56
Scaling alone	0.60 ± 0.14 *p*=0.001	0.29 ± 0.13 *p*=0.047	-0.15 ± 0.19 *p*=0.45
One-way ANOVA	F=3.95 ***p*=0.013***	F=0.44 ***p*=0.726**	F=0.06 ***p*=0.979**

* The only significantly different pair of differences was Tetracycline fibre - scaling. The corresponding 95% confidence interval for the Tukey test was (0.16 , 1.33).

Table 3.15 'Between-visit' changes for attachment level; The early change was the change from baseline untill week 6, the intermediate change was calculated by subtraction of the 3 month attachment level from the 6 week attachment level. The late change was calculated by subtraction of the 6 month attachment level from the 3 month attachment level. Mean ± standard error of the mean are shown. The p values in each cell represent paired t-test in changes occurred during the interval between the 2 consecutive visits. The data are subject derived (patient as the unit of analysis).

Between-visit change	Between baseline & week 6 (early change)	Between week 6 & month 3 (intermediate change)	Between month 3 & month 6 (late change)
Tetracycline fibre	0.75 ± 0.12 $p<0.001$	-0.04 ± 0.10 $p=0.65$	-0.00 ± 0.21 $p=0.99$
Minocycline gel	0.45 ± 0.14 $p=0.006$	0.05 ± 0.08 $p=0.56$	0.00 ± 0.14 $p=0.98$
Metronidazole gel	0.57 ± 0.15 $p=0.002$	0.13 ± 0.10 $p=0.21$	-0.15 ± 0.11 $p=0.19$
Scaling alone	0.20 ± 0.11 $p=0.032$	0.19 ± 0.08 $p=0.038$	-0.01 ± 0.14 $p=0.94$
One-way ANOVA	F=2.43 **$p=0.076$**	F=1.24 **$p=0.305$**	F=0.23 **$p=0.877$**

3.1.11 The percentage of sites which showed pocket depth reduction/attachment gain above 3 x standard deviation of measurements

A series of site-specific analysis were performed on the pocket depth and attachment level data. For a site to be considered as successfully treated, a pre-defined threshold had to have been surpassed.

The results of the preliminary study on the intra-examiner reproducibility of the measurements of pocket depth and attachment level (section 2.2.3) revealed that the standard deviation of differences between duplicated readings were 0.42 mm for the probing pocket depth and 0.34 mm for the attachment level measurements using the same method and examiner used in the present study. Three times standard deviations of the probing depth and attachment level measurements i.e. 1.26 mm and 1.02 mm respectively were selected as a threshold for treatment success. Since the resolution of duplicate readings of the Florida Probe is 0.1 mm the thresholds were set at least 1.3 mm and 1.1 mm for the pocket depth reductions and the attachment level changes.

Table 3.16 shows the chi-square analysis for the successfully treated sites as defined above for the pocket depth reduction and attachment gain. The analysis of pocket depth data indicated that at the 6 week and 3 month visits no significant difference existed between the

groups (chi-square= 7.259, and 4.713 respectively, not significant). At the 6 week visit, the percentage of sites with reduced pocket depth ≥ 1.3 mm was 41.2% for the scaling plus tetracycline fibre group, 35.7% for the scaling plus minocycline gel group, 34.6% for the scaling plus metronidazole gel group, and 17.7% for the scaling alone group. The corresponding values for the 3 month follow-up visit were 49.0%, 30.4%, 45.5% and 37.3%. However, at the 6 month visit a significant difference was found between the groups (chi-square=9.674, p=0.025). The follow-up analysis using the 2x2 contingency tables revealed the presence of significant difference between the scaling plus tetracycline fibre group and the scaling plus minocycline gel group (chi-square = 8.84, p<0.001) and between the scaling plus metronidazole gel group and the scaling plus minocycline gel group (chi-square = 4.38, p=0.036).

Similar analysis on the attachment level data indicated that there was a significant difference between the groups at 6 week (chi-square = 13.028, p<0.01). Follow-up analysis using multiple 2x2 contingency tables indicated that there was significant difference between the scaling plus tetracycline fibre group and the scaling alone (chi-square = 13.028, p<0.001) and between the scaling plus metronidazole gel group and the scaling alone group (chi-square = 10.827, p=0.001). The percentages of 'attachment gainer' sites were 31.4%, 19.6%, 30.9% and 5.9% in the

Table 3.16 Number and percentage of sites which showed pocket depth reduction > 1.26 mm and sites which showed attachment gain > 1.02 mm (3x standard deviation of differences of pocket depth and attachment level measurements respectively determined in pilot study, section 2.2.3) at follow-up visits. N.S: not significant.

Treatment	Pocket reduction > 1.26 mm	Attachment gain > 1.02 mm
6 week:		
Tetracycline fibre	21 41.2%	16 31.4%
Minocycline gel	20 35.7%	11 19.6%
Metronidazole gel	19 34.6%	17 30.9%
Scaling alone	9 17.7%	3 5.9%
	χ^2= 7.259 N.S	**χ^2= 13.028, p<0.01†**
3 month:		
Tetracycline fibre	25 49.0%	13 25.5%
Minocycline gel	17 30.4%	12 21.4%
Metronidazole gel	25 45.5%	18 32.7%
Scaling alone	19 37.3%	11 21.6%
	χ^2= 4.713, N.S	**χ^2= 2.423, N.S**
6 month:		
Tetracycline fibre	26 51.0%	18 35.3%
Minocycline gel	13 23.2%	8 14.3%
Metronidazole gel	23 41.8%	17 30.9%
Scaling alone	17 33.3%	15 29.4%
	χ^2= 9.674, p=0.025†	**χ^2= 6.907, N.S**

† Significantly different pairs:
Tetracycline fibre-Minocycline gel, chi-square=8.84, p<0.001.
Metronidazole gel-Minocycline gel, chi-square= 4.38, p=0.036.

‡ Significantly different pairs:
Tetracycline fibre-Scaling alone, chi-square=13.028, p<0.001.
Metronidazole gel-Scaling alone, chi-square=10.827, p=0.001.

tetracycline fibre, metronidazole gel, minocycline gel and the scaling alone groups respectively. However, no significant association was found between the treatments and the proportion of 'attachment gainer' sites at 3 month and 6 month visits-(chi-square=2.423, and 7.127 respectively, not significant). The percentage of 'attachment gainer' sites at the 3 month visit were 25.5%, 21.4%, 32.7%, and 21.6% for the adjunctive tetracycline fibre, minocycline gel, metronidazole gel and the scaling alone groups respectively. The corresponding figures for the 6 month visit were 35.3%, 14.3%, 30.9% and 29.4% respectively.

3.1.12 Bleeding on probing

Table 3.17 shows the number and percentage of sites within each treatment group which showed bleeding on probing at baseline, 6 week, 3 month and 6 month visits. Chi-square analysis for the baseline data was not possible due to the presence of too many small expected frequencies in the table. However, the Kruskal-Wallis test revealed no significant difference between groups (p=0.891) at the baseline.

The Cochran-Q test revealed that within each treatment group, there were significant differences between the bleeding scores of different time points (p<0.001, Table 3.18). Follow-up analysis indicated that at the 6 week visit post-treatment, the bleeding on probing scores

Table 3.17 Number and percentage of sites with bleeding on probing at baseline, 6 week, 3 month, and 6 month visits among the 4 treatment groups.

Time point	Tetracycline fibre	Minocycline gel	Metronidazole gel	Scaling alone
baseline	47 (92.2%)	51 (91.1%)	48 (87.3%)	49 (96.1%)
6 weeks	21 (41.2%)	32 (57.1%)	29 (52.7%)	32 (62.7%)
3 months	18 (35.3%)	22 (39.3%)	24 (43.6%)	30 (58.8%)
6 months	23 (45.1%)	31 (55.4%)	31 (56.4%)	24 (47.1%)
Total	51	56	55	51

Table 3.18 Test for the association of bleeding on probing with the time point and probability values obtained by comparisons across different time points for each treatment group. Probability values in the first row of the table were obtained using the Cochran-Q test (baseline v.s 6 week v.s 3 month v.s 6 month), and the p values in the remaining cells were obtained using the McNemar's test. If it was necessary, Fisher's exact probability for the McNemar's tables were calculated.

Treatment	Tetracycline fibre	Minocycline gel	Metronidazole gel	Scaling alone
Cochran-Q test	***p*<0.001**	***p*<0.001**	***p*<0.001**	***p*<0.001**
Baseline v.s 6 week	*p*<0.001*	*p*<0.001*	*p*<0.001*	*p*<0.001*
Baseline v.s 3 month	*p*<0.001*	*p*<0.001*	*p*<0.001*	*p*<0.001*
Baseline v.s 6 month	*p*<0.001*	*p*<0.001*	*p*<0.001*	*p*<0.001*
6 week v.s 3 month	*p*=0.648	*p*=0.052	*p*=0.424	*p*=0.134
6 week v.s 6 month	*p*=0.845	*p*=1.00	*p*=0.839	*p*=0.791
3 month v.s 6 month	*p*=0.383	*p*=0.078	*p*=0.189	*p*=0.327

* Due to the assumptions of the McNemar test not being held, the *p* values marked with (*) were obtained using the Fisher's exact test.

Table 3.19 Chi-square analysis on association of improvement in bleeding on probing at the 6 week, 3 month and 6 month time points with treatment.

BOP status	Tetracycline fibre n=51	Minocycline gel n=56	Metronidazole gel n=55	Scaling alone n=51	Total n=213
		6 week change:			
Improved	26 (51.0%)	22 (39.3%)	22 (40.0%)	18 (35.3%)	88 (41.3%)
Other	25 (49.0%)	34 (60.7%)	33 (60.0%)	33 64.7%)	125 (58.7%)
	Chi-square = 2.826, df = 3 (not significant)				
		3 month change:			
Improved	29 (56.9%)	31 (55.4%)	27 (49.1%)	25 (49.0%)	112 (52.6%)
Other	22 (43.1%)	25 (44.6%)	28 (50.9%)	26 (51.0%)	101 (47.4%)
	Chi-square = 1.076, df = 3 (not significant)				
		6 month change:			
Improved	27 (52.9%)	24 (42.9%)	20 (36.4%)	20 (39.2%)	91 (42.7%)
Other	24 (47.1%)	32 (57.1%)	35 (63.6%)	31 (60.8%)	122 (57.3%)
	Chi-square = 3.342, df = 3 (not significant)				

Table 3.20 Chi-square analysis on association of frequency of sites which bled on probing on all the 3 follow-up occasions with treatment.

Treatment	sites which bled on all 3 follow-up occasions	Other sites
Tetracycline fibre	6 (11.8%)	45 (88.2%)
Minocycline gel	13 (23.2%)	43 (76.8%)
Metronidazole gel	9 (16.4%)	46 (83.6%)
Scaling alone	13 (25.6%)	38 (74.5%)
Total	41 (19.2%)	172 (80.8%)
Chi-square = 3.977, df = 3 (not significant)		

decreased highly significantly in all treatment groups ($p<0.001$, Fisher's exact test for paired data). This statistically significant improvement was sustained at this level ($p<0.001$) in all treatment groups until the end of 6 month follow-up study. All treatment groups showed some further reduction in bleeding on probing at the 3 month visit compared to the 6 week visit. While in the scaling alone group the frequency of bleeding on probing continued to decrease until the 6 month visit, all 3 adjunctive antimicrobial treatment groups showed some degrees of relapse in the frequency of bleeding on probing at the 6 month visit compared to the 3 month visit. However, further follow-up analysis for each treatment group indicated that no significant changes took place after week 6 in any of the treatment groups (Table 3.18).

Table 3.19 displays the frequency of sites whose bleeding on probing improved. There were no statistical differences between the treatments at any follow-up visit (chi-square = 2.826 for the 6 week, 1.076 for the 3 month and 3.342 for 6 month visits, not significant). However, at any time point the improvement in the scaling plus tetracycline fibre group was the greatest with more than half of the sites showing improvement, closely followed by the other 3 treatments.

Table 3.20 shows the frequency of sites which in spite of treatment, bled on probing at all 3 follow-up occasions.

The scaling alone group showed the highest percentage of such sites (25.6%), followed by the scaling plus minocycline gel group (23.2%), scaling plus metronidazole gel group (16.4%), and finally scaling plus tetracycline fibre group (11.8%). However, the chi-square analysis revealed no statistical association between the treatments and prevalence of sites with bleeding on probing on 3 consecutive visits post-treatment (chi-square = 3.977, not significant).

3.1.13 Suppuration

Table 3.21 displays the number and frequency of the sites which showed suppuration at the baseline, 6 weeks, 3 month and 6 month visits. All sites showed reduction in the frequency of suppurating sites after treatment. In the scaling plus tetracycline fibre group the frequency of suppurating sites reached zero at 6-week and 3-month visits, although 1 site showed suppuration at the 6 month visit. This site had no suppuration at the baseline (Diagram 3.22). At the 3 month visit, the reduction in the suppuration remained significant only in the scaling plus tetracycline fibre group (p=0.016). In fact, in the scaling alone group a complete return towards the baseline value occurred at 3 months. In the scaling plus minocycline gel and scaling plus metronidazole gel group some degrees of relapse were observed. At the 6 month visit, the scores had almost returned to the original scores in the 2 scaling alone and scaling plus

Table 3.21 Number and percentage of sites with suppuration at baseline, 6 week, 3 month, and 6 month visits among the 4 treatment groups.

Time point	Tetracycline fibre	Minocycline gel	Metronidazole gel	Scaling alone
baseline*	6 11.8%	4 7.2%	8 14.5%	8 15.7%
6 weeks	0 0%	2 3.6%	1 1.9%	2 3.9%
3 months	0 0%	3 5.4%	4 7.3%	8 15.7%
6 months	1 2%	1 1.8%	7 12.7%	9 17.7%
Total number of sites	51	56	55	51

	Scaling & tetracycline fibre (total n=51)	Scaling & minocycline gel (total n=56)	Scaling & metronidazole gel (total n=55)	Scaling alone (total n=51)
Baseline	●●●●●●○	●●●●○○○	●●●●●●●●○○○	●●●●●●●●○○○○○
6 week	○○○○○○○	●●○○○○○	○○○○○○○○●○○	●○○○○○○○●○○○○
3 month	○○○○○○○	●○○○○●●○	●●●○○○○○○●○○	●●●●○○○○○●●●●○
6 month	○○○○○○●	○○○○○○●	●●●●●○○○○●●	●●●○●○○○●●●●●

Diagram.3.22 Diagram of sites which showed at least one episode of suppuration. ●: suppuration, ○: no suppuration. Symbols on the same vertical position represent the suppuration status of a single site throughout the study period.

Table 3.23 Matrix of pairwise comparisons to test the association of the treatments with the frequency of suppuration episodes before and after treatment using the Fisher's exact test (*p* values are given).

	Tetracycline fibre	Minocycline gel	Metronidazole gel
Minocycline gel	*p*= 0.076		
Metronidazole gel	*p*= 0.048*	*p*= 0.654	
Scaling alone	*p*= 0.009*	*p*= 0.844	*p*= 0.686

* significant difference

Table 3.24 Matrix of pairwise comparisons to test the association of the treatments with the presence of suppuration at 6 week, 3 month and 6 month visit using the Fisher's exact test (*p* values are given).

6 weeks:

	Tetracycline fibre	Minocycline gel	Metronidazole gel
Minocycline gel	*p*= 0.272		
Metronidazole gel	*p*= 0.519	*p*= 0.382	
Scaling alone	*p*= 0.247	*p*= 0.369	*p*= 0.363

3 month:

	Tetracycline fibre	Minocycline gel	Metronidazole gel
Minocycline gel	*p*= 0.272		
Metronidazole gel	*p*= 0.068	*p*= 0.278	
Scaling alone	*p*= 0.003*	*p*= 0.057	*p*= 0.099

6 month:

	Tetracycline fibre	Minocycline gel	Metronidazole gel
Minocycline gel	*p*= 0.779		
Metronidazole gel	*p*= 0.038*	*p*< 0.001*	
Scaling alone	*p*= 0.007*	*p*< 0.001*	*p*> 0.300†

* significant difference

† Chi-square test was indicated, $\chi^2 = 0.500$, df= 1

metronidazole gel groups. However, in the other 2 groups i.e. scaling plus tetracycline fibre and scaling plus minocycline gel groups, only one site in each group had suppuration.

The number of suppuration episodes throughout the whole follow-up period were calculated and compared among the groups (Table 3.23). The scaling plus tetracycline fibre group (with one episode) was found to have significantly less suppuration episodes than the scaling plus metronidazole gel group (12 episodes) (p=0.048) and scaling alone group (20 episodes) (p=0.009). The difference between the scaling plus tetracycline fibre and scaling plus minocycline gel groups (6 episodes) failed to reach statistical significance (p=0.076). All other comparisons were clearly insignificant.

Since performance of between-treatment comparisons using 2x4 contingency tables would not be valid due to the presence of small expected counts in too many cells, the pair-wise 2x2 comparisons between the treatments were performed for each follow-up visit (Table 3.24). No significant difference between any pair of treatments was revealed at week 6. At the 3 month visit, the difference between the scaling plus tetracycline fibre and the scaling alone groups were statistically significant (p=0.003). However, at the 6 month visit, the scaling plus tetracycline fibre group was significantly different

from the scaling alone (p=0.007) and the scaling plus metronidazole gel (p=0.038) groups. The scaling plus minocycline gel group was also significantly different from the scaling alone (p<0.001) and the scaling plus metronidazole gel (p<0.001) groups.

3.1.14 The Plaque Index

Table 3.25 shows the frequency distribution of plaque scores across the treatment groups at baseline, 6 week, 3 month and 6 month visits. There was no significant difference between the treatment groups at any time point (p=0.570, p=0.259, p=0.465, and p=0.739 for the baseline, 6 week, 3 month and the 6 month visits respectively, Kruskal-Wallis test). The mean plaque scores were generally low at all time points and was always less than 1 for any treatment group at any occasion and the majority of sites had plaque score of 0 or 1 and very few sites showed a plaque score of 3. Nevertheless, a slight increase in the plaque scores for all treatment groups was observed towards the end of the study.

3.1.15 The Modified Gingival Index

The frequency distribution of the Modified Gingival Index (MGI) data is displayed in Table 3.26. There was no significant difference between the groups at the baseline (p=0.122). The MGI scores decreased significantly at 6 week in all groups (Table 3.27) and remained significantly lower than the baseline scores until the end of study.

Table 3.25 Frequency distribution of Plaque score data among the 4 treatment groups at baseline, 6 week, 3 month and 6 month visits. Mean ± standard error of the mean (SEM) and medians are shown. Probability values for the Kruskal-Wallis test are also displayed.

Time point	Plaque score	Tetracycline fibre n=51	Minocycline gel n=56	Metronidazole gel n=55	Scaling alone n=51
Baseline	score 0	30	30	27	21
	score 1	16	20	23	25
	score 2	5	4	5	5
	score 3	0	2	0	0
	mean ± SEM	0.51 ± 0.09	0.61 ± 0.10	0.60 ± 0.09	0.69 ± 0.09
	median	0	0	1	1
	p= 0.570 (Kruskal-Wallis test)				
6 week	score 0	28	24	31	19
	score 1	14	23	16	20
	score 2	19	9	7	11
	score 3	0	0	1	1
	mean ± SEM	0.63 ± 0.11	0.73 ± 0.10	0.60 ± 0.11	0.88 ± 0.11
	median	0	1	0	1
	p= 0.259 (Kruskal-Wallis test)				
3 month	score 0	16	26	26	24
	score 1	27	14	22	20
	score 2	7	13	7	7
	score 3	1	3	0	0
	mean ± SEM	0.86 ± 0.10	0.87 ± 0.13	0.65 ± 0.09	0.67 ± 0.10
	median	1	1	1	1
	p= 0.465 (Kruskal-Wallis test)				
6 month	score 0	17	25	21	20
	score 1	21	20	22	21
	score 2	12	10	11	9
	score 3	1	1	1	1
	mean ± SEM	0.94 ± 0.11	0.77 ± 0.11	0.86 ± 0.11	0.82 ± 0.11
	median	1	1	1	1
	p= 0.739 (Kruskal-Wallis test)				

Table 3.26 Frequency distribution of Modified Gingival score data among the 4 treatment groups at baseline, 6 week, 3 month and 6 month visits. Mean ± standard error of the mean (SEM) and medians are shown. Probability values for Kruskal-Wallis test are also displayed.

Time point	Plaque score	Tetracycline fibre n=51	Minocycline gel n=56	Metronidazole gel n=55	Scaling alone n=51
Baseline	score 0	0	5	1	0
	score 1	5	7	11	5
	score 2	27	30	31	28
	score 3	19	12	12	18
	score 4	0	2	0	0
	mean	2.27	1.98	1.98	2.25
	± SEM	± 0.09	± 0.12	± 0.10	± 0.09
	median	2	2	2	2
		p= **0.122** (Kruskal-Wallis)			
6 week	score 0	7	4	6	1
	score 1	28	22	17	14
	score 2	16	30	28	29
	score 3	0	0	4	7
	score 4	0	0	0	0
	mean	1.18	1.46	1.55	1.82
	± SEM	± 0.09	± 0.10	± 0.10	± 0.10
	median	1	2	2	2
		p= **0.001** (Kruskal-Wallis)			
3 month	score 0	25	6	4	2
	score 1	24	23	20	19
	score 2	2	24	26	26
	score 3	51	3	5	4
	score 4	0	0	0	0
	mean	1.55	1.42	1.58	1.63
	± SEM	± 0.08	± 0.11	± 0.10	± 0.10
	median	2	1	2	2
		p= **0.631** (Kruskal-Wallis)			
6 month	score 0	3	3	5	1
	score 1	24	23	15	16
	score 2	23	22	26	25
	score 3	1	8	9	9
	score 4	0	0	0	0
	mean	1.43	1.63	1.71	1.82
	± SEM	± 0.09	± 0.11	± 0.12	± 0.10
	median	1	2	2	2
		p= **0.107** (Kruskal-Wallis)			

When the changes in the MGI were tested for between-treatment differences, a significant difference was found between groups at the 6 week (p=0.002) and 6 month (p=0.012) visits. However, there was no significant difference between groups at the 3 month visit (p=0.340). The post-hoc comparisons using multiple Mann-Whitney U tests (Table 3.28) indicated that at the 6 week visit, the scaling plus tetracycline fibre group had a significantly greater improvement in MGI scores than the scaling plus minocycline gel (p=0.03), scaling plus metronidazole gel (p=0.003), and scaling alone (p=0.002) groups. The remaining differences were not significant. At the 6 month visit, similar to 6 week visit, the differences between the scaling plus tetracycline fibre group and the other 3 groups were significant (p=0.013 for scaling plus minocycline gel, p<0.001 for the scaling plus metronidazole gel, and p=0.036 for the scaling alone groups). In addition, the improvement in the scaling alone group was significantly higher than the scaling plus metronidazole gel group (p=0.012). However, when their actual scores at the follow-up visits instead of their improvement from baseline were compared across the treatments, there was no significant difference between the groups at the 3 and 6 month visits (p=0.631 and p=0.107 respectively) and only the 6 week comparison revealed a significant difference (p=0.001).

Table 3.27 Change in the MGI(modified Gingival Index) data from baseline among the 4 treatment groups at 6 week, 3 months and 6 month visits. Mean and 95% confidence interval and median of change in MGI are displayed. Probability values within each cell represent the paired comparison between the baseline and the corresponding follow-up visit using the Wilcoxon test. Probability values in the last row of the table represent between treatment comparisons at each time point for changes in MGI using the Kruskal-Wallis test.

Treatment		6 week visit	3 month visit	6 month visit
Tetracycline fibre	mean	1.10	0.73	0.84
	CI†	(0.85 - 1.35)	(0.51 - 0.94)	(0.59 - 1.10)
	med.‡	1	1	1
		***p*<0.001**	***p*<0.001**	***p*<0.001**
Minocycline gel	mean	0.52	0.55	0.36
	CI	(0.22 - 0.82)	(0.30 - 0.81)	(0.12 - 0.60)
	med.	1	1	0
		***p*=0.003**	***p*<0.001**	***p*=0.011**
Metronidazole gel	mean	0.43	0.31	0.27
	CI	(0.20 - 0.56)	(0.07 - 0.56)	(0.03 - 0.51)
	med.	0	0	0
		***p*=0.002**	***p*=0.005**	***p*= 0.036**
Scaling alone	mean	0.43	0.63	0.43
	CI	(0.23 - 0.64)	(0.39 - 0.87)	(0.21 - 0.65)
	med.	0	1	1
		***p*=0.001**	***p*<0.001**	***p*=0.002**
Kruskal-Wallis *p* value		***p*=0.002**	***p*= 0.340**	***p*= 0.012**

† CI : 95% confidence interval
‡ med. : median

Table 3.28 Matrix of pair-wise comparisons of 6 week improvement and 6 month improvement in the Modified Gingival Index (MGI) between treatments using the Mann-Witney U test.

	A. 6 week change:		
	Tetracycline fibre	**Minocycline gel**	**Metronidazole gel**
Minocycline gel	p*=0.008		
Metronidazole gel	p=0.001	p=0.624	
Scaling alone	p<0.001	p=0.613	p=0.942

	B. 6 month change:		
	Tetracycline fibre	**Minocycline gel**	**Metronidazole gel**
Minocycline gel	p=0.018		
Metronidazole gel	p=0.0018	p=0.438	
Scaling alone	p=0.049	p=0.623	p=0.150

* After the Bonferroni correction for the multiple comparisons the threshold for a significant probability value was p<0.020.

3.1.16 The effect of smoking status on the treatment outcome

Table 3.29 shows the improvement in the pocket depth and attachment level among the smoker and non-smoker patients, regardless of their treatments. At all the follow-up occasions the improvement in the non-smoker patients were greater than the smoker patients for both probing depth and attachment level data. The 2-sample independent t-tests showed that for pocket depth reductions, the 6 week and 6 month improvements were significantly greater in the non-smokers than in the smokers (p=0.019 and p=0.010 respectively). However, the 3 month comparison did not show a significant difference (p=0.090). The changes in attachment levels were not significantly different at any time points (p=0.84, p=0.59 and p=0.095 for the 6 week, 3 month and the 6 month visits respectively).

At baseline the smokers had a significantly lower GCF volume than the non-smokers (p=0.023). In addition, the plaque levels of the smokers was not greater than that of the non-smokers.

Since the data indicated that smoking may have had an influence on the treatment outcome (Table 3.29), it was decided to test this effect in a more integrated approach together with the other factors i.e. the treatment (which was of most interest) and the baseline pocket depth (which was shown to have an important effect on outcome).

Therefore, the changes in the pocket depth and attachment levels were tested in the General linear model (GLM) procedures. The smoking status and treatment were the main factors with 2 and 4 factor levels respectively and the baseline pocket depth was a continuous covariate. The initial model testings were performed by including all the individual effects together with the 2-order and the 3-order interactions. If the 3-order interaction was not significant, the model would be tested without the 3-order interaction. Similarly, the 2-order interactions were removed one by one if they were not significant. Therefore, the final model included only the main factors and the significant interaction terms (if any). Tables 3.30 to 3.35 show this analysis for the pocket depth reduction and attachment gain data at the 6 week, 3 month and the 6 month visits. These series of analysis consistently revealed that a significant interaction existed between the smoking status and the baseline pocket depth at all time points for both the pocket depth reduction and the attachment gain. Due to the presence of such an interaction the interpretation of either of the individual effects of baseline pocket depth and the smoking status was not possible. However, since the treatment was not a component of a significant interaction (except for the 3 month pocket depth reductions) the probability values obtained for the treatment were interpretable (with one exception) as the significance of the treatment effect alone. The treatment effect was

significant only for the 6 week pocket depth reductions and the 6 month pocket depth reductions (p=0.002 and p=0.042 respectively). The presence of interaction between the smoking and the baseline pocket depth indicated that the effect of having deeper pocket depth on the treatment outcome was different for the smokers and non-smokers. Further inspection of such interaction was performed by plotting the changes and the least square lines for the smokers and non-smokers against the initial pocket depth. Figures 3.3 to 3.8 display the overlaid regression lines for the smokers and non-smokers for probing depth and attachment level changes. The Figures consistently indicated that while in the non-smokers there was a linear relationship between the baseline pocket depth and the changes in the pocket depth and attachment level, in the smoker patients, very weak relationships existed between the baseline pocket depth and the changes in the pocket depth or attachment level.

Table 3.36 shows the improvements in the pocket depth and attachment level for each time point among the treatment groups. The results indicated that the probability values obtained for between-treatment comparisons are generally smaller in the non-smoker sub-group than the smoker sub-group (with the exception of 6 month pocket depth changes). In other words, in the non-smoker sub-group, the treatment effect was often stronger than it was in the smoker sub-group.

Table 3.29 The change in pocket depth (PD) and attachment level (AL), the plaque Index, and the GCF volume in all patients broken down by their smoking status (regardless of their treatment type). Mean ± standard error of the mean are shown. *p* values were obtained using 2-sample t-tests between smokers and non-smokers data.

a) pocket depth reduction and attachment gain (mm)

smoking status	PD change 6 week	PD change 3 month	PD change 6 month	AL change 6 week	AL change 3 month	AL change 6 month
Smokers (n=28)	0.76 ±0.11	0.92 ±0.14	0.72 ±0.12	0.50 ±0.09	0.55 ±0.10	0.40 ±0.11
Non-smokers (n=26)	1.14 ±0.12	1.28 ±0.15	1.28 ±0.17	0.52 ±0.10	0.63 ±0.12	0.71 ±0.13
2-sample t-test	p=0.019	p=0.090	p=0.010	p=0.845	p=0.594	p=0.069

b) Plaque Index :

smoking status	PI Baseline	PI 6 week	PI 3 month	PI 6 month
Smokers (n=28)	0.55 ±0.08	0.65 ±0.11	0.73 ±0.12	0.86 ±0.12
Non- smokers (n=26)	0.65 ±0.10	0.68 ±0.11	0.89 ±0.10	0.83 ±0.10
2-sample t-test	p=0.44	p=0.84	p=0.31	p=0.85

c) GCF volume (µl)

smoking status	GCF Baseline	GCF 6 week	GCF 3 month	GCF 6 month
Smokers (n=28)	0.41 ±0.03	0.41 ±0.03	0.42 ±0.03	0.40 ±0.03
Non- smokers (n=26)	0.51 ±0.03	0.44 ±0.03	0.49 ±0.03	0.48 ±0.03
2-sample t-test	p=0.023	p=0.48	p=0.088	p=0.055

Table 3.30 Analysis of Variance (GLM) for change in pocket depth at 6 week visit. The final model includes only the main effects and the significant interaction terms (PD0=baseline pocket depth, TREAT=treatment, SMOKE=smoking status).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SMOKE	1	1.9553	0.5585	0.5585	2.44	0.125
TREAT	3	4.3680	3.9084	1.3028	5.70	0.002
PD0	1	1.4883	1.7738	1.7738	7.76	0.008
SMOKE*PD0	1	0.9314	0.9314	0.9314	4.07	0.049
Error	47	10.7472	10.7472	0.2287		
Total	53	19.4901				

Table 3.31 Analysis of Variance (GLM) for change in attachment levels at 6 week visit. The final model includes only the main effects and the significant interaction terms (PD0=baseline pocket depth, TREAT=treatment, SMOKE=smoking status).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SMOKE	1	0.0107	0.8623	0.8623	4.63	0.037
TREAT	3	1.6677	1.3501	0.4500	2.42	0.078
PD0	1	1.5014	1.7913	1.7913	9.62	0.003
SMOKE*PD0	1	0.9502	0.9502	0.9502	5.10	0.029
Error	47	8.7514	8.7514	0.1862		
Total	53	12.8814				

Table 3.32 Analysis of Variance (GLM) for change in pocket depth at 3 month visit. The final model includes only the main effects and the significant interaction terms(PD0=baseline pocket depth, TREAT=treatment, SMOKE=smoking status).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SMOKE	1	1.7375	3.7783	3.7783	10.13	0.003
TREAT	3	3.0439	2.2470	0.7490	2.01	0.127
SMOKE*TREAT	3	4.1079	3.8286	1.2762	3.42	0.025
PD0	1	1.9282	2.7270	2.7270	7.31	0.010
SMOKE*PD0	1	4.6265	4.6265	4.6265	12.41	0.001
Error	44	16.4067	16.4067	0.3729		
Total	53	31.8506				

Table 3.33 Analysis of Variance (GLM) for change in attachment level at 3 month visit. The final model includes only the main effects and the significant interaction terms (PD0 = baseline pocket depth, TREAT = treatment, SMOKE = smoking status).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
SMOKE	1	0.0964	2.1881	2.1881	7.89	0.007
TREAT	3	0.7016	0.4356	0.1452	0.52	0.668
PD0	1	1.0730	1.4931	1.4931	5.38	0.025
SMOKE*PD0	1	2.3784	2.3784	2.3784	8.58	0.005
Error	47	13.0322	13.0322	0.2773		
Total	53	17.2815				

Table 3.34 Analysis of Variance (GLM) for change in pocket depth at 6 month visit. The final model includes only the main effects and the significant interaction terms (PD0 = baseline pocket depth, TREAT = treatment, SMOKE = smoking status).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	3	3.2003	2.9209	0.9736	2.95	0.042
SMOKE	1	4.9117	5.0262	5.0262	15.25	0.000
PD0	1	3.9940	5.3107	5.3107	16.12	0.000
SMOKE*PD0	1	6.5268	6.5268	6.5268	19.81	0.000
Error	47	15.4872	15.4872	0.3295		
Total	53	34.1200				

Table 3.35 Analysis of Variance (GLM) for change in attachment level at 6 month visit. The final model includes only the main effects and the significant interaction terms (PD0=baseline pocket depth, TREAT=treatment, SMOKE=smoking status).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TREAT	3	0.4707	0.4577	0.1526	0.51	0.674
SMOKE	1	1.5283	1.5939	1.5939	5.37	0.025
PD0	1	3.2411	3.8732	3.8732	13.06	0.001
SMOKE*PD0	1	2.0881	2.0881	2.0881	7.04	0.011
Error	47	13.9438	13.9438	0.2967		
Total	53	21.2720				

Table 3.36 The probing pocket depth and attachment level changes across the treatment groups at different time points, broken down by the smoking status of the patients. Probability values in the last column represent the analysis of covariance to test the treatment effect with the baseline pocket depth as covariate. The data are patient derived. Mean and the standard error of the mean are displayed.

	Smoking status	Tetracycline fibre	Minocycline gel	Metronidazole gel	Scaling alone	*p* value
Pocket depth change (mm) (6 week)	N†	1.73 ± 0.27	0.98 ± 0.17	1.18 ± 0.12	0.80 ± 0.22	**0.010**
Pocket depth change (6 week)	S‡	1.11 ± 0.19	0.78 ± 0.20	0.63 ± 0.31	0.37 ± 0.11	**0.128**
Attachment gain (mm) (6 week)	N	0.93 ± 0.22	0.43 ± 0.17	0.64 ± 0.19	0.18 ± 0.15	**0.035**
Attachment gain (6 week)	S	0.64 ± 0.13	0.47 ± 0.21	0.48 ± 0.25	0.36 ± 0.17	**0.758**
Pocket depth change (3 month)	N	1.60 ± 0.37	0.76 ± 0.22	1.64 ± 0.28	1.07 ± 0.26	**0.030**
Pocket depth change (3 month)	S	1.37 ± 0.24	1.03 ± 0.26	0.40 ± 0.24	0.69 ± 0.31	**0.098**
Attachment gain (3 month)	N	0.82 ± 0.34	0.37 ± 0.14	0.91 ± 0.26	0.42 ± 0.17	**0.228**
Attachment gain (3 month)	S	0.63 ± 0.15	0.60 ± 0.22	0.43 ± 0.28	0.50 ± 0.19	**0.919**
Pocket depth change (6 month)	N	1.67 ± 0.41	0.99 ± 0.21	1.31 ± 0.36	1.22 ± 0.37	**0.490**
Pocket depth change (6 month)	S	1.21 ± 0.27	0.69 ± 0.11	0.64 ± 0.14	0.19 ± 0.27	**0.028**
Attachment gain (6 month)	N	1.06 ± 0.19	0.49 ± 0.25	0.68 ± 0.31	0.73 ± 0.26	**0.394**
Attachment gain (6 month)	S	0.48 ± 0.27	0.53 ± 0.10	0.40 ± 0.27	0.24 ± 0.20	**0.798**

† N: non-smokers ‡ S: Smokers

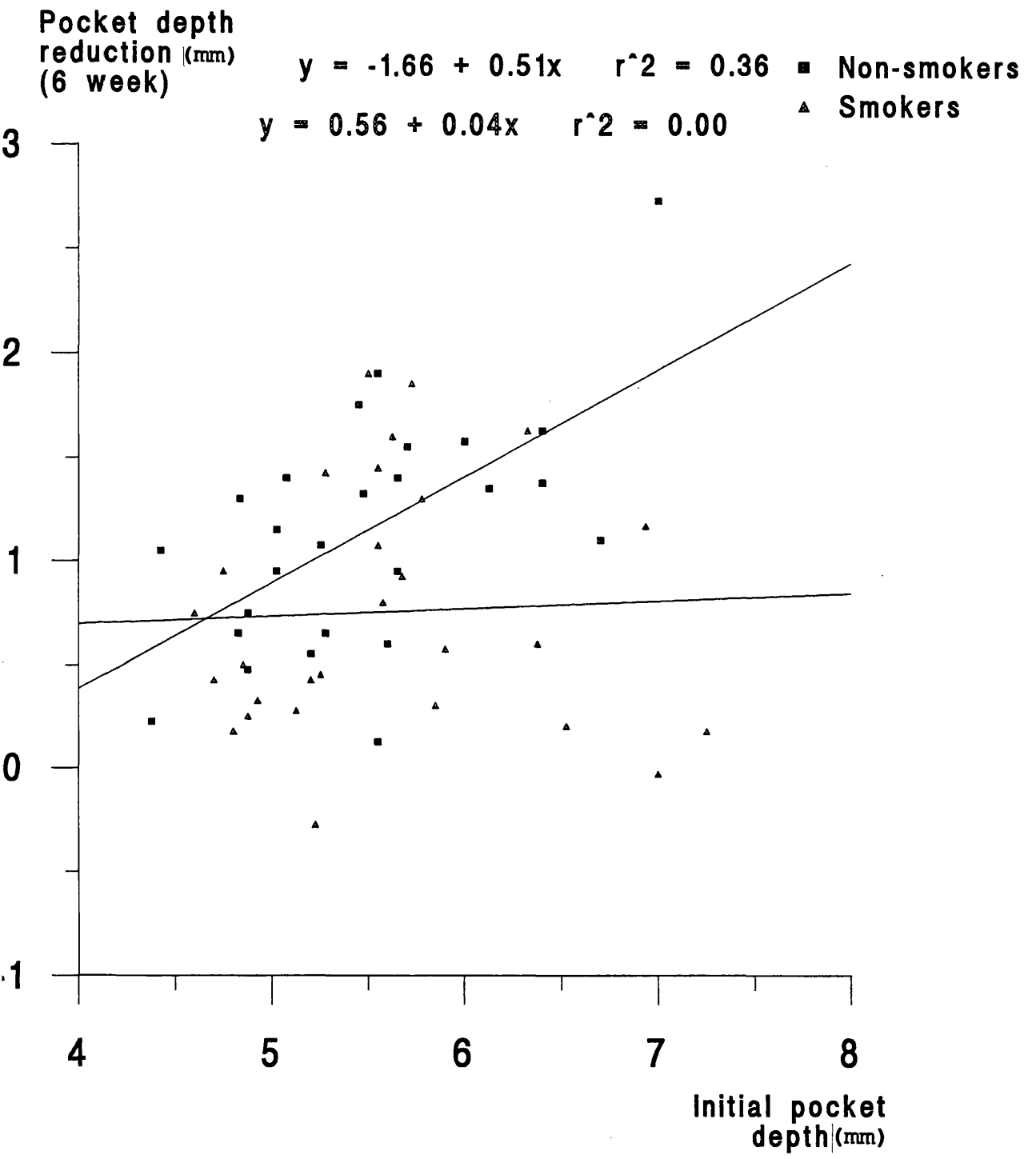

Fig. 3.3 Plot and regression lines of pocket depth reduction (6-week) against the initial pocket depth for the smokers and non-smokers.

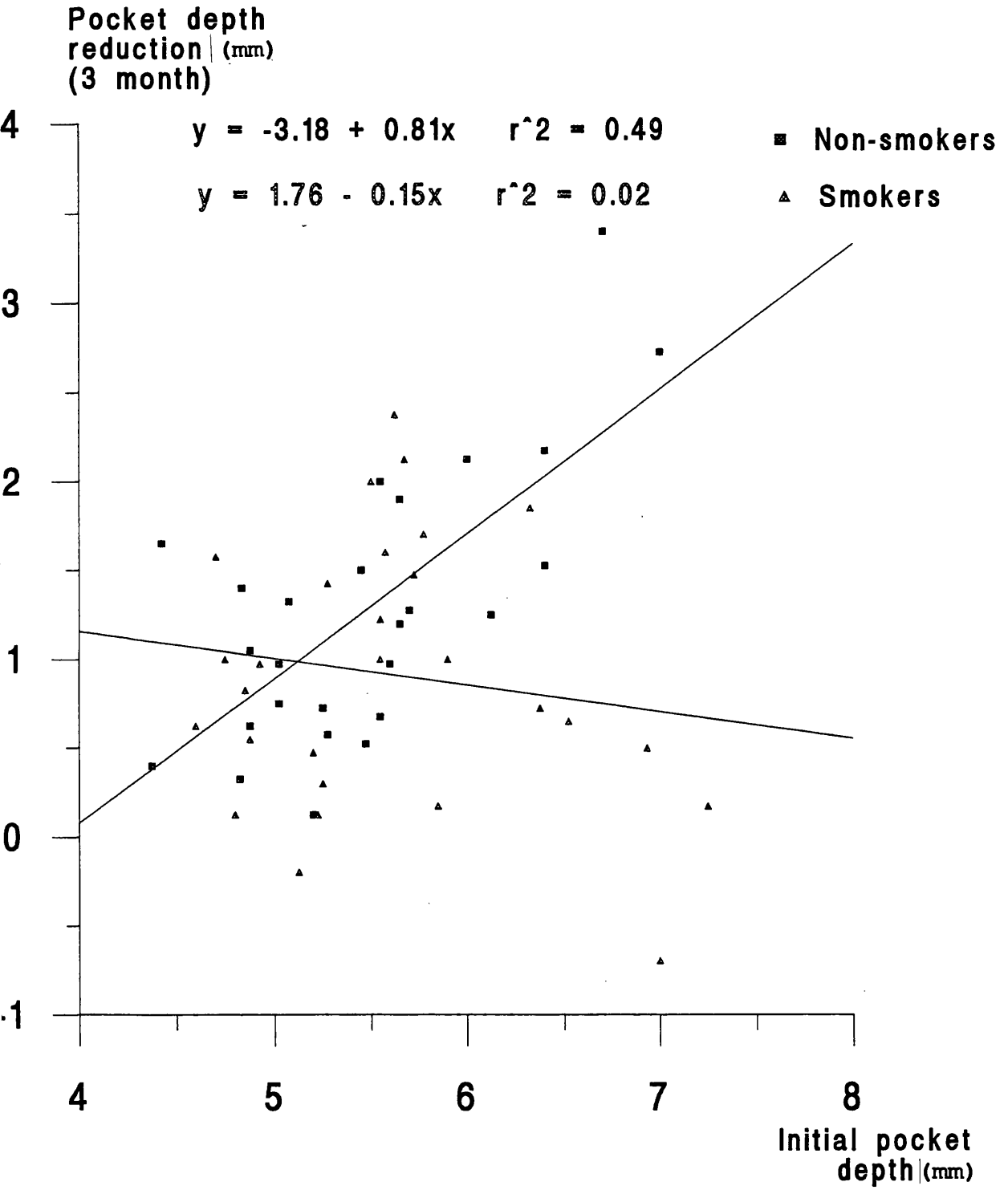

Fig. 3.4 Plot and regression lines of pocket depth reduction (3-month) against the initial pocket depth for the smokers and non-smokers.

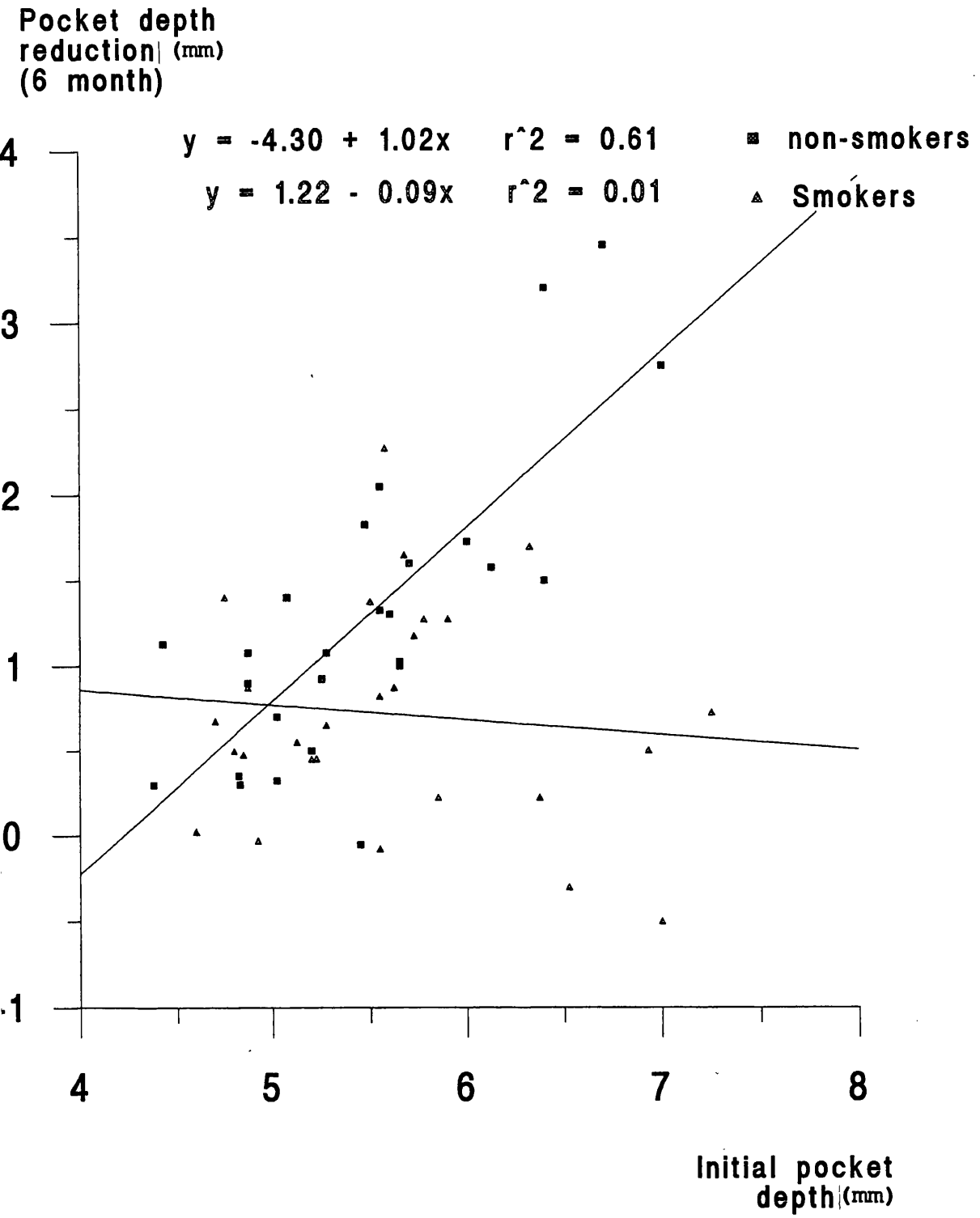

Fig. 3.5 Plot and regression lines of pocket depth reduction (6-month) against the initial pocket depth for the smokers and non-smokers.

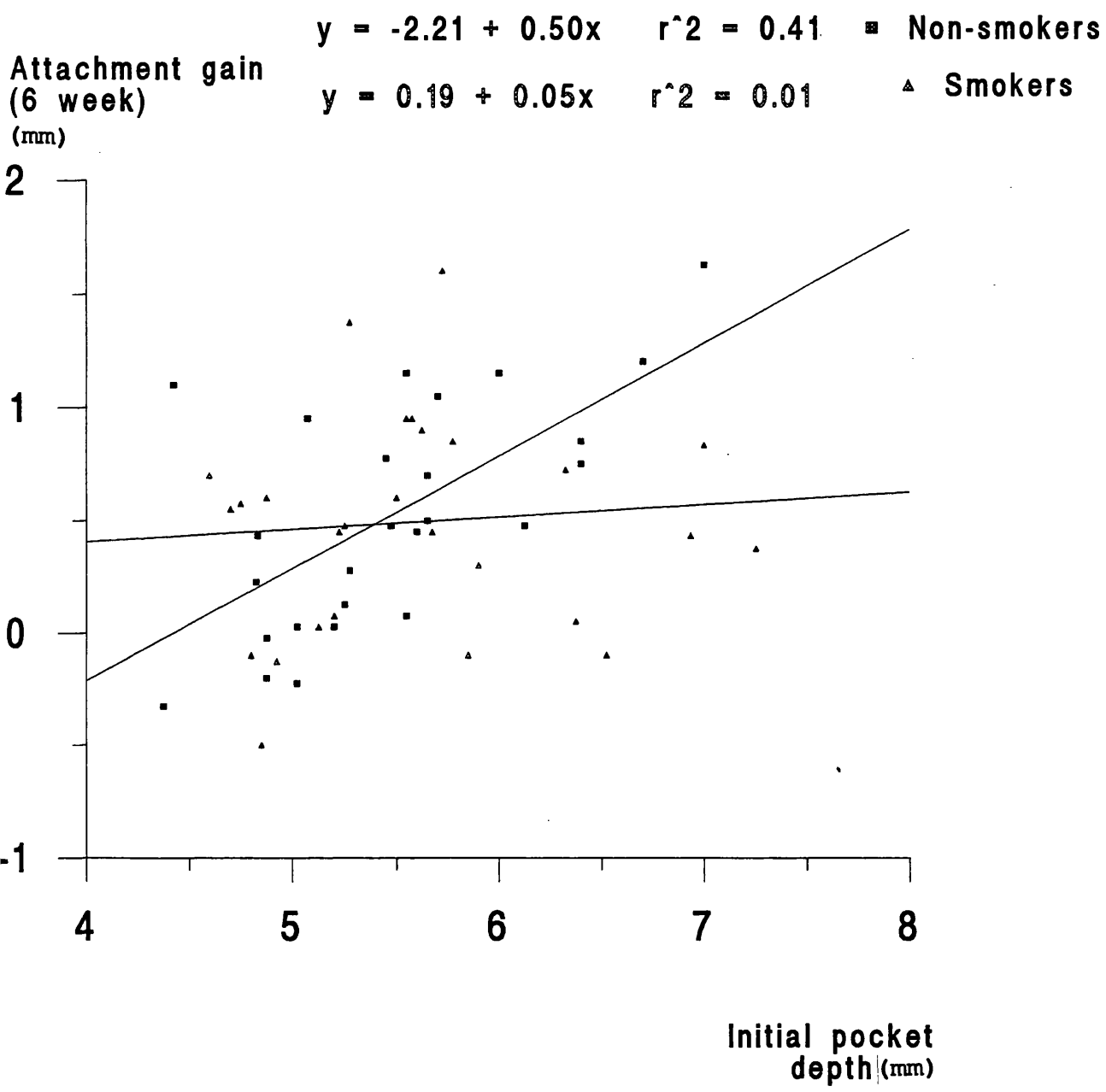

Fig. 3.6 Plot and regression lines of attachment level changes (6-week) against the initial pocket depth for the smokers and non-smokers.

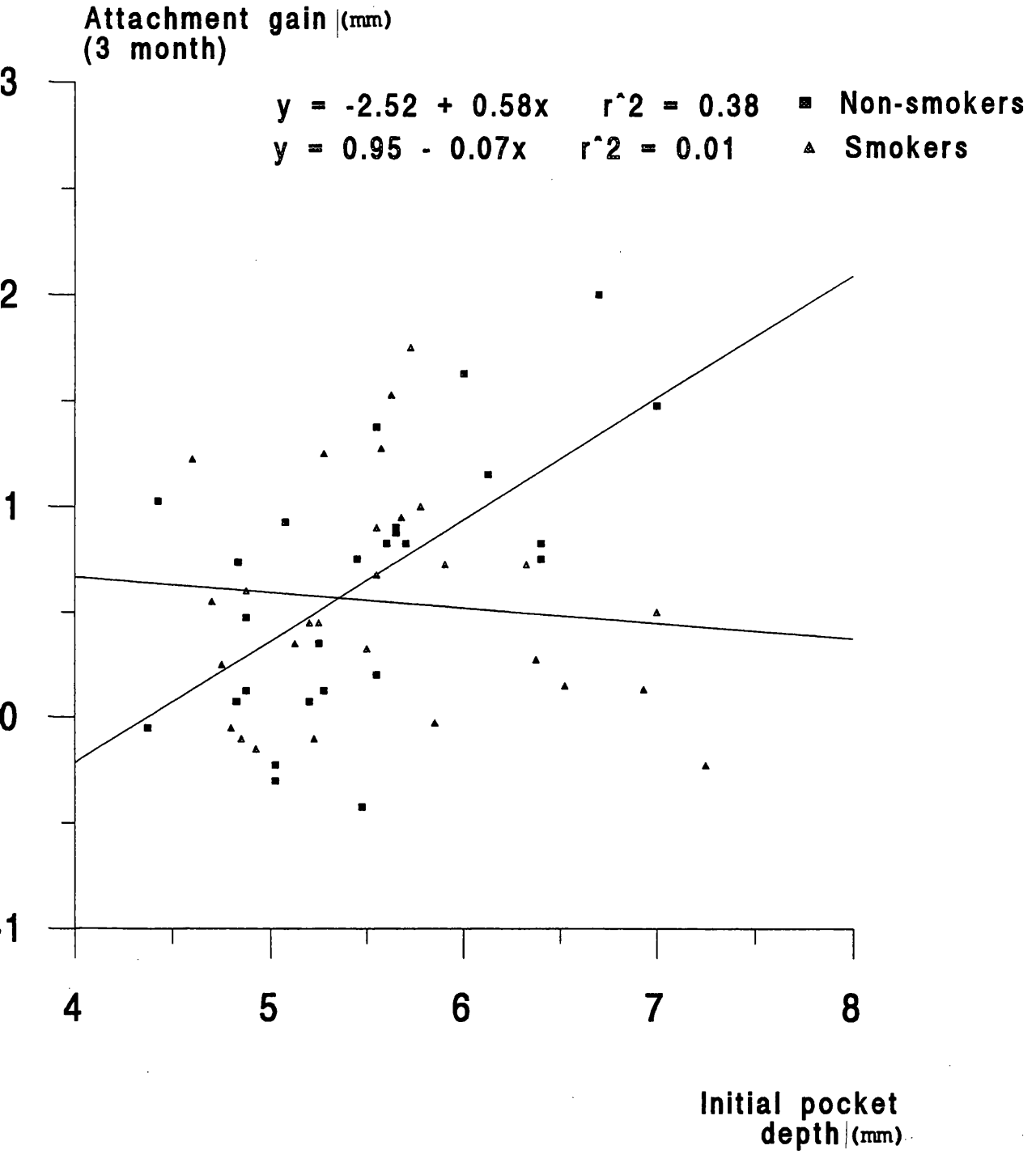

Fig. 3.7 Plot and regression lines of attachment level changes (3-month) against the initial pocket depth for the smokers and non-smokers.

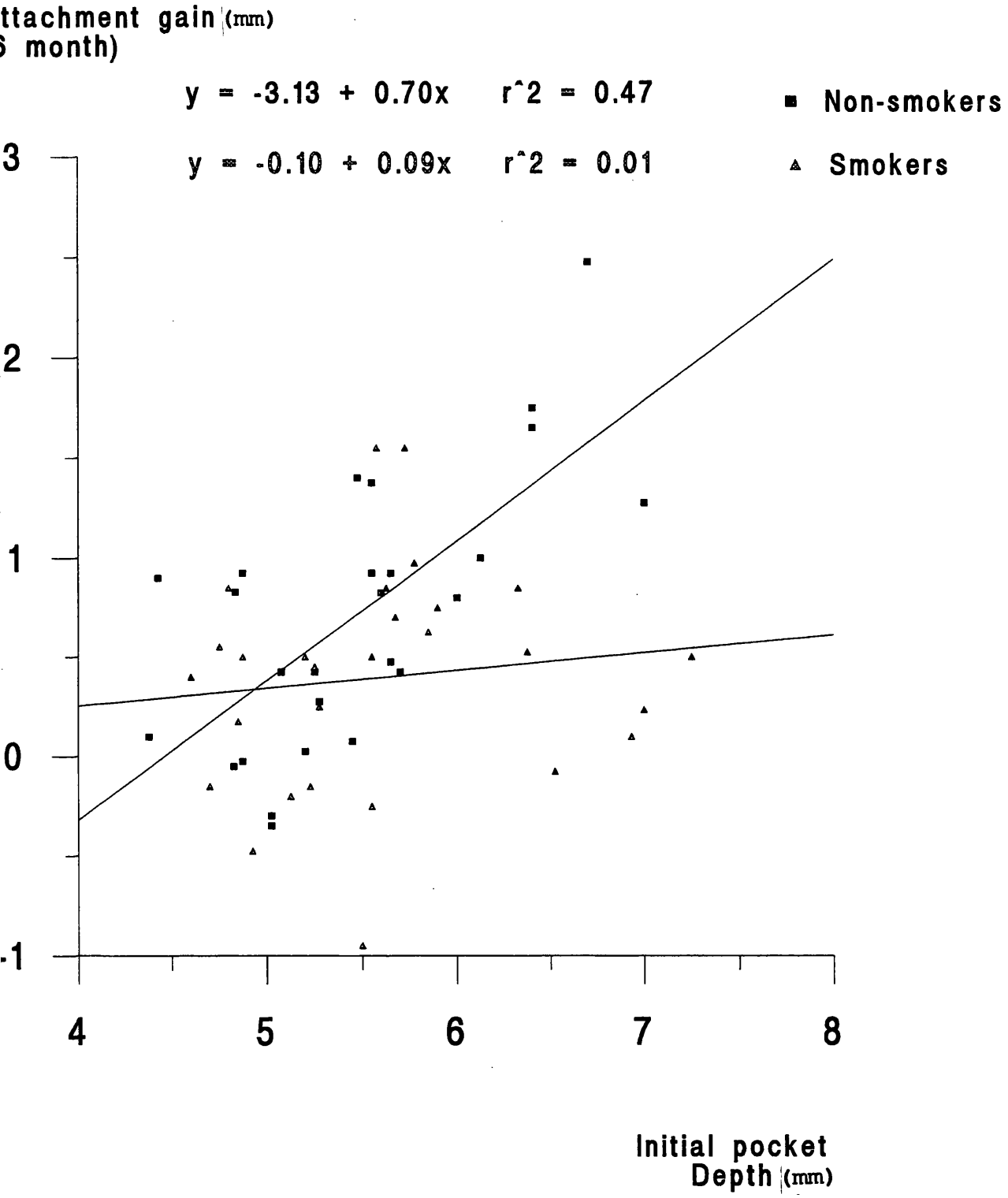

Fig. 3.8 Plot and regression lines of attachment level changes (6-month) against the initial pocket depth for the smokers and non-smokers.

3.1.17 The effect of previous history of periodontal surgery on pocket depth reduction

Twenty-three percent of all sites had been previously treated by means of periodontal flap surgery. The distribution of such sites among the 4 treatment groups were similar and ranged from 18% to 24% (chi-square=1.079, d.f.=3, not significant). Table 3.37 shows the pocket depth reduction at various follow-up time points for sites with or without previous surgery. At all 3 follow-up visits, the improvement in pocket depth was greater at sites which did not have previous surgery. This difference almost approached the statistically significance level at the 3-month follow-up visit (p=0.057).

3.1.18 The gingival crevicular fluid volume (GCF)

The GCF volumes are shown in Table 3.38. There was no difference in the GCF volumes at the baseline between treatment groups (p=0.308). After 6 week there was a significant reduction in the GCF volume in the scaling plus tetracycline fibre group (p=0.031). However, this reduction did not remain significant at the 3 month and 6 month visits. In the remaining 3 groups no significant change took place in the GCF volumes after the treatment.

3.1.19 Adverse effects

No serious adverse effect such as toxic or allergic reactions was observed or reported by any of the patients.

Table 3.37 Pocket depth reduction at sites with different history of previous periodontal surgery. Mean, standard error of the mean, 95% confidence interval for difference and the *p* value for the two-sample t test are shown.

Time point	Mean ± SEM (mm)		95% CI & *p* value
	No Surgery (n=164)	surgery (n=49)	
6-week	0.95 ± 0.08	0.82 ± 0.15	-0.20 , 0.48 *p*=0.430
3-month	1.16 ± 0.09	0.81 ± 0.16	-0.01 , 0.72 *p*=0.057
6-month	1.08 ± 0.10	0.76 ± 0.15	-0.03 , 0.69 *p*=0.076

Table 3.38 Gingival crevicular volumes (μl) obtained at baseline, 6 week, 3 month and 6 month visits among the 4 treatment groups. Mean ± standard error of the mean are displayed. Probability values were obtained by the paired t-tests between the baseline and a follow-up time point volumes for each treatment.

Treatment	Baseline†	6 week	3 month	6 month
Tetracycline fibre	0.49 ± 0.04	0.38 ± 0.04 **p=0.031**	0.46 ± 0.04 **p=0.56**	0.43 ± 0.03 **p=0.12**
Minocycline gel	0.43 ± 0.04	0.41 ± 0.04 **p=0.62**	0.49 ± 0.04 **p=0.28**	0.45 ± 0.04 **p=0.73**
Metronidazole gel	0.41 ± 0.04	0.45 ± 0.04 **p=0.40**	0.39 ± 0.04 **p=0.78**	0.42 ± 0.05 **p=0.71**
Scaling alone	0.51 ± 0.05	0.46 ± 0.05 **p=0.23**	0.49 ± 0.05 **p=0.70**	0.46 ± 0.04 **p=0.27**

† No difference existed between treatments at baseline (p=0.31).

Table 3.39 Adverse effects in each treatment group

Treatment	Symptom	Sign
Tetracycline fibre	Slight pain on 1st day of fibre therapy (1 patient)	Slight redness upon fibre removal (5 patients)
Minocycline gel	Bad taste on 1st day of gel application (1 patient) Gingival tingling on the 1st day of gel application (1 patient)	Nil
Metronidazole gel	Bad taste on 1st day of gel application (2 patients)	Nil
Scaling **alone**	Nil	Nil

However, mild and transient effects were observed/reported (Table 3.39).

3.1.20 Time taken for the treatments

Each study tooth and their adjacent teeth were scaled and root planed under local anaesthesia for approximately 5 minutes each. In addition, all deep pockets within the mouth were scaled and root planed under local anaesthesia. A minimum of one 45-60 minute visit was necessary for this purpose. An additional visit was used if required. The gel therapy normally took 10 minutes per session. The average time taken for fibre insertion was 8.2 minutes per tooth with a range of 5-20 minutes. The removal of fibres took 11.0 minutes per patient.

3.1.21 Sites requiring intervention

At the 3 month visit, one site in the scaling and root planing alone group developed an abscess, showed severe attachment loss and caused discomfort for the patient. This tooth was given additional treatment which comprised scaling and root planing. The 6 month measurements were taken for this site. This site showed some further attachment loss at the 6 month visit. It is normally recommended to exclude the data of such sites from the analysis and instead, to analyse the frequency of excluded sites among the treatment groups as an outcome variable (Imrey, 1986). It is most likely that the additional treatment at this site has slowed down the attachment loss

and therefore, the analysis of data without exclusion of this site would have diluted the difference between the efficacy of the 4 treatments. On the other hand, since the changes in probing depth and attachment level of this site at the 6 month visit, i.e., after the additional treatment, were still far inferior to the mean of remaining sites which received the same treatment, exclusion of this site from the 6 month analysis or from the entire analysis would have further diluted the real differences between the treatments. Therefore, recognising the slight diluting effect resulting from the additional treatment of this site, it was decided to keep this site in the analysis.

3.2 An evaluation of the effect of Nd:YAG laser on calculus, cementum and dentine, an *in vitro* study

The electron microscopic features of laser treated tissue seemed very typical. These features include melting down of the tooth material and calculus. The mineral seemed to melt and then re-solidify. This resulted in a very porous surface. The size of pores ranged from 1 to 20 microns. In those specimens where severe damage was evident, a hole had been created which was lined with melted material.

Increasing the energy setting and duration of the laser, generally resulted in increase in the severity of damage to calculus, cementum and dentine, although it was observed that cementum and dentine were not affected using low energy settings and short durations. On the other hand, calculus was more or less affected using all energy settings and durations.

3.2.1 Calculus

Table 3.40 shows the degree of damage for each of the 94 treated sites on 32 teeth, along with the mean damage score for each tooth.

Figure 3.9 shows the relationship between the log of the total energy input and the mean damage score. The least squares linear regression line was:

mean damage score = 0.925 + 1.54 $\log_{10}$(total energy input)

The slope of the line was significantly different from 0 ($p<0.001$) and $r^2=66\%$. A plot of residuals against the fitted values from this regression indicated that a straight line gave an adequate fit to the data. Thus there is convincing evidence that higher total energy input leads to a greater mean damage score.

The effect of the power setting of the laser, the number of pulses per second and the length of time on the mean damage score were further investigated by a three-way analysis of variance. The results are shown in Table 3.41. None of the interaction terms are significant. However the main effects of power setting, number of pulses per second and length of time are contributing independently to the mean damage score in an additive way.

A consistent observation was that specimens with complete evaporation of calculus also showed some degree of damage to the underlying cementum (Fig. 3.10), however, because of the variability in calculus thickness which could not be controlled, this damage to the underlying cementum was not scored in the present study.

3.1.2 Cementum and dentine

Tables 3.42 and 3.43 show the degree of damage to the cementum and dentine specimens. Large variability was

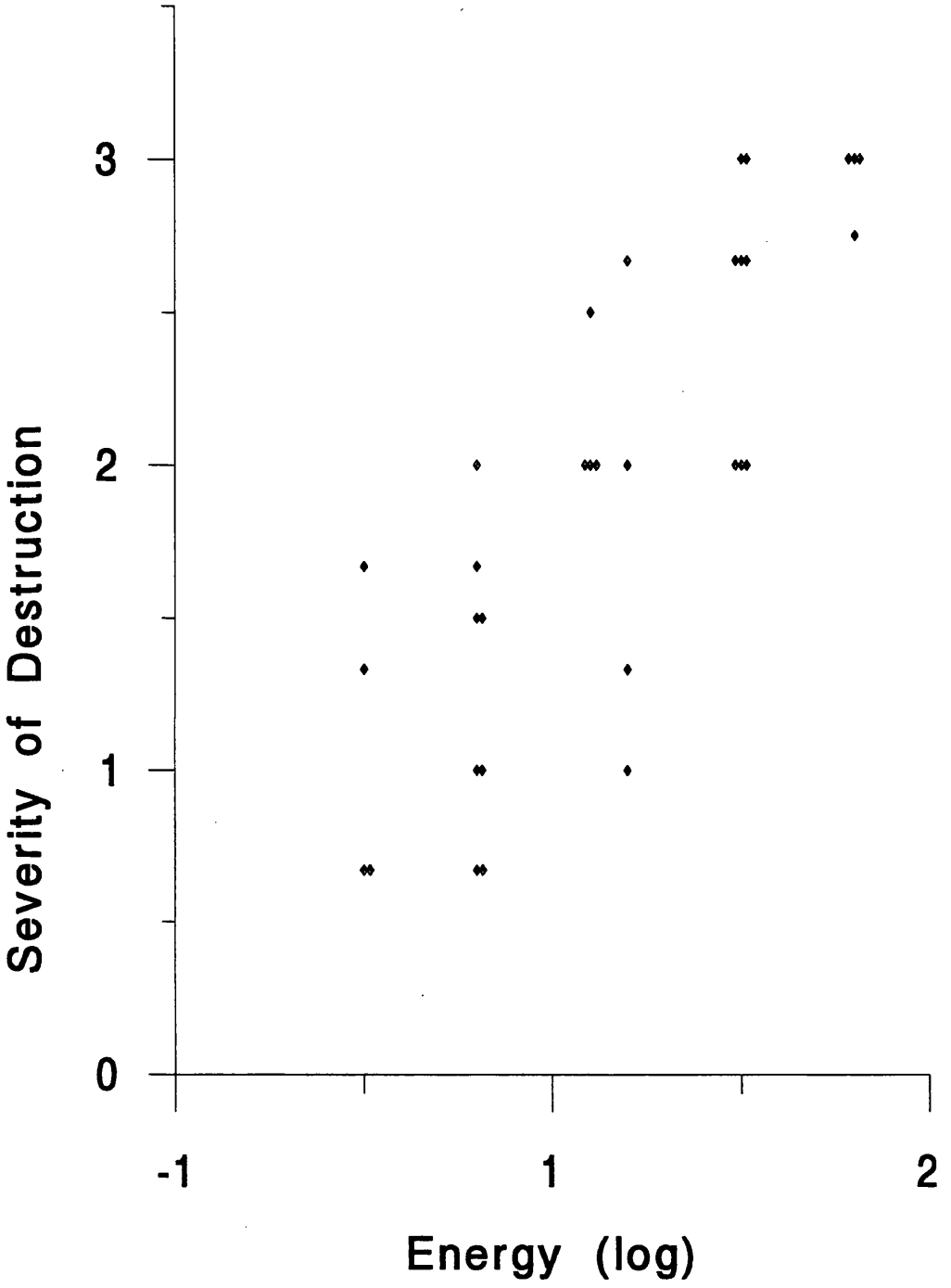

Fig. 3.9 The relationship between the total laser energy input (log) and the damage score on subgingival calculus.

LASER SETTING		50mJ. 10pps 1 second (Group 1)					50mJ. 10pps 5 seconds (Group 2)					100mJ. 10pps 1 second (Group 3)					100mJ. 10pps 5 seconds (Group 4)					50mJ. 20pps 1 second (Group 5)					50mJ. 20pps 5 seconds (Group 6)					100mJ. 20pps 1 second (Group 7)					100mJ. 20pps 5 seconds (Group 8)				
SPECIMEN NUMBER		1	9	17	25	T	2	10	18	26	T	3	11	19	27	T	4	12	20	28	T	5	13	21	29	T	6	14	22	30	T	7	15	23	31	T	8	16	24	32	T
DEGREE OF PHYSICAL CHANGE	0			* *	*	3			*	*	2		*			1					0	*		* *		3				*	1	*				1					0
	1	*	* *		* *	5		*	*		2	* *	*	*	* * *	7					0	* *	*			3					0					0					0
	2	* *	*	*		4	*	*	*	* *	5		*	*		2		*	*	* * *	5		*	*	* * *	5	*	* * *			4	*	* * *	* * *	*	8		*			1
	3					0	* *	*			3	*				1	* * *	* * *	* *		7					0	* *	*	* * *	*	7	* *			*	3	* * *	* * *	* * *	* *	11
NO. OF TREATMENTS		3	3	3	3		3	3	3	3		3	3	2	3		3	3	3	3		3	2	3	3		3	3	3	3		4	3	3	2		3	4	3	2	
MEAN OF DAMAGE		1.67	1.33	0.67	0.67		2.67	2.0	1.0	1.33		1.67	1.0	1.5	1.0		3.0	2.67	2.67	2.0		0.67	1.5	0.67	2.0		2.67	2.0	3.0	2.0		2.0	2.0	2.0	2.5		3.0	3.0	2.75	3.0	

Table 3.40 Degree of physical changes at each site (laser treatment of subgingival calculus).

Table 3.41 Three-way analysis of variance of the effect of power setting, number of pulses per second and length of time on the mean damage score.

source	d.f.	Sum of squares	F	p
Power	1	3.07	13.3	<0.001
Pulses/sec	1	1.96	8.4	0.008
Time	1	7.91	34.2	<0.001
Power * Pulses	1	0.08	0.3	0.57
Power * Time	1	0.03	0.1	0.74
Pulses * Time	1	0.00	0.0	0.93
Power*Pulses*Time	1	0.52	2.3	0.15
Error	24	5.56		
Total	31			

Laser settings and durations		50 mJ 10 pps 1 s			50 mJ 10 pps 5 s			100 mJ 10 pps 1 s			100 mJ 10 pps 5 s			50 mJ 20 pps 1 s			50 mJ 20 pps 5 s			100 mJ 20 pps 1 s			100 mJ 20 pps 5 s		
Specimens		D	E	F	D	E	F	D	E	F	D	E	F	D	E	F	D	E	F	D	E	F	D	E	F
Damage score	0	*	*		*			*			*			*	*		*	*		*	*		*		
	1			*																					
	2					*				*						*						*			
	3						*		*			*	*						*					*	*

Table 3.42 Distribution of damage scores by different laser treatments on dentine.

Laser settings and durations		50 mJ 10 pps 1 s			50 mJ 10 pps 5 s			100 mJ 10 pps 1 s			100 mJ 10 pps 5 s			50 mJ 20 pps 1 s			50 mJ 20 pps 5 s			100 mJ 20 pps 1 s			100 mJ 20 pps 5 s		
Specimens		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Damage score	0	*	*	*	*	*	*	*					*	*	*	*	*			*		*				
	1									*	*								*							
	2								*			*									*					
	3																	*					*	*	*

Table 3.43 Distribution of damage scores by different laser treatments on cementum.

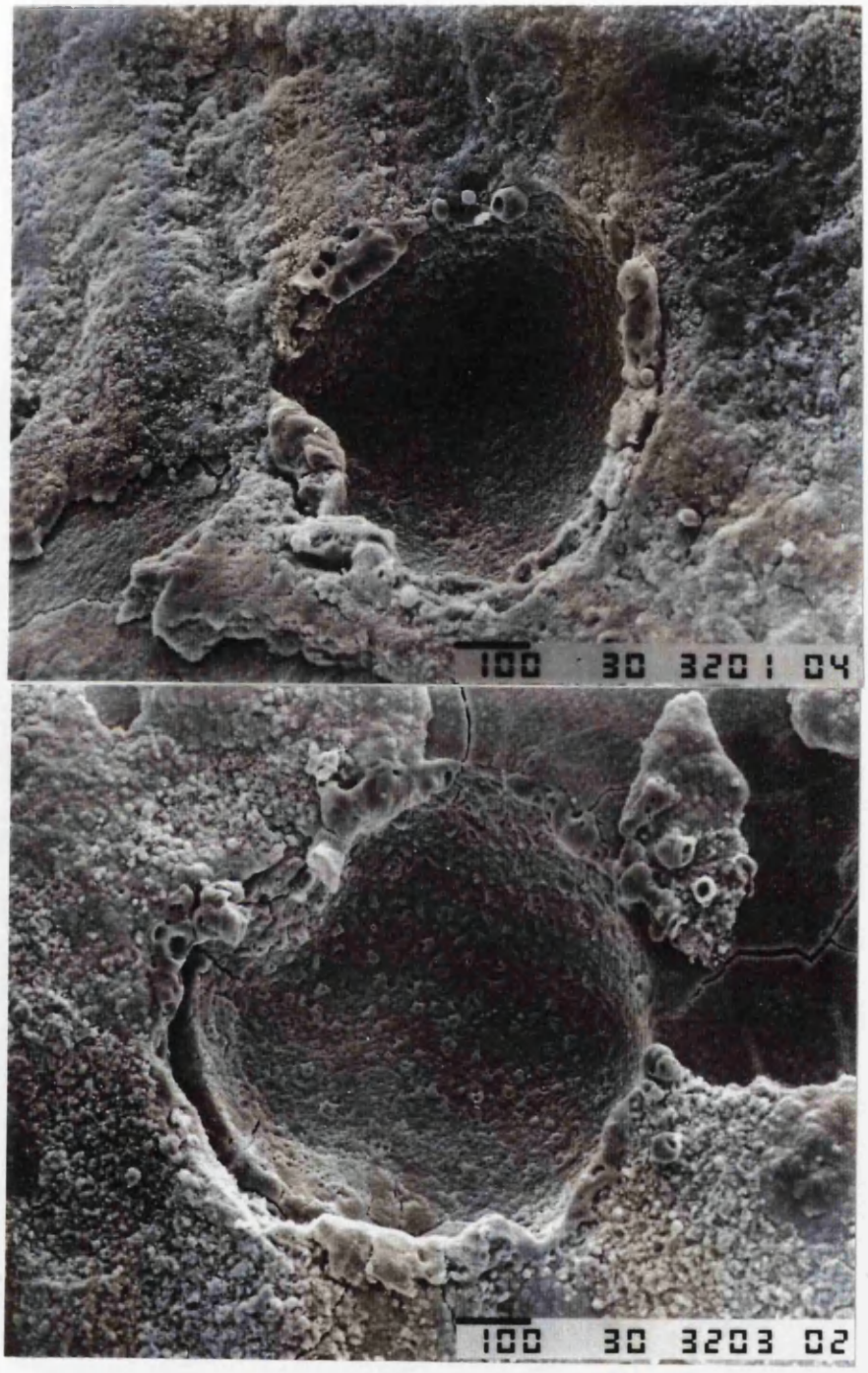

Fig. 3.10 Laser treated subgingival calculus. The laser has evaporated not only the calculus but also the underlying cementum layer. (top: 50 mJ, 10 pps, 1 sec; bottom: 100 mJ, 20 pps, 1 sec; next page: 100 mJ, 20 pps, 1 sec).

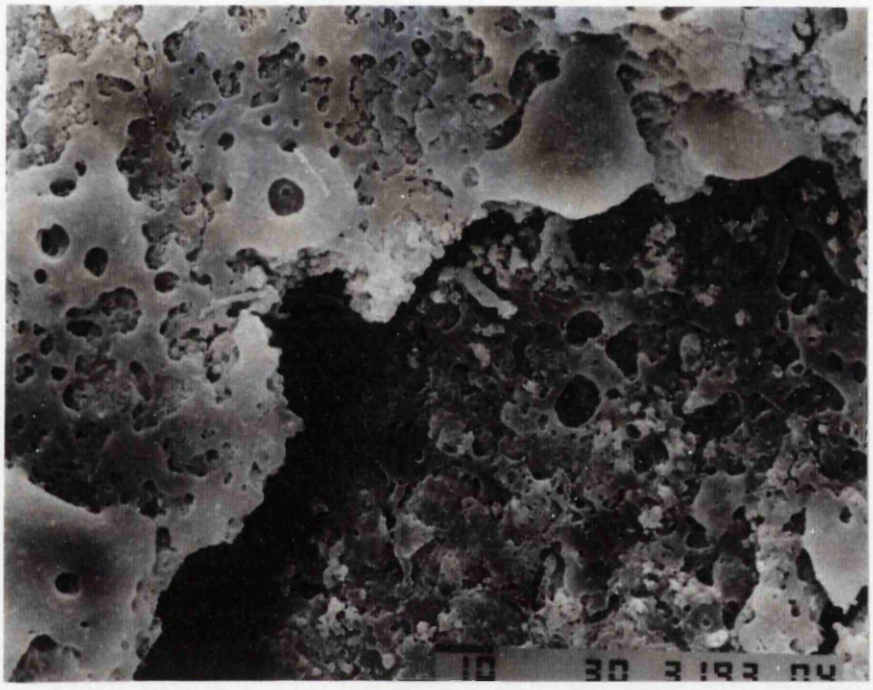

Fig. 3.10 (continued)

observed between different teeth, for both cementum and dentine specimens. For instance, tooth D was affected by none of the laser exposures, whereas tooth F was affected by all of the power settings employed.

3.3 Evaluation of the Nd:YAG laser in periodontal pocket therapy

3.3.1 Plaque Index and Modified Gingival Index

Overall the plaque index scores recorded for all sites were relatively low during the study (0.6 at baseline and 0.37 at 6-weeks). There was a slight reduction in plaque scores at the 6-week visit compared to baseline for all four groups, although only the control group showed a significant reduction (p=0.043 Wilcoxon's test). Friedman's test on changes in plaque scores among the 4 groups showed no significant differences (p=0.94) (Table 3.44). The gingival index also was slightly reduced in all groups over time with the highest reduction being recorded in the scaling group (mean reduction 0.5) followed by the 50mJ laser, 80mJ laser and control groups with mean reductions of 0.26, 0.05 and 0.05 respectively. No significant difference was found in changes of gingival scores among the 4 groups (p=0.29) (Table 3.45).

3.3.2 Probing pocket depth

All groups showed a reduction in pocket depth at the 6-week visit compared to baseline (Table 3.46), but only the scaling group showed a significant reduction (p<0.001, paired t-test). Repeated measures analysis of variance on differences of the 4 groups proved to be significant (p=0.003). In addition, Duncan's multiple range test

Table 3.44 Plaque Index at baseline and six-week visits among the different treatment groups. Mean ± Standard error of the mean are displayed.

Treatment	Baseline	6 week	Change†
Scaling and root planing	0.65 ± 0.18	0.50 ± 0.17	0.15 ± 0.21
Laser '50 mJ'	0.80 ± 0.21	0.45 ± 0.14	0.35 ± 0.23
Laser '80 mJ'	0.50 ± 0.14	0.40 ± 0.15	0.10 ± 0.12
Untreated control	0.45 ± 0.15	0.15‡ ± 0.11	0.30 ± 0.13

† p=0.939, Friedman's test.
‡ Significantly different from baseline, p=0.043, Wilcoxon's test.

Table 3.45 Modified Gingival Index at baseline and six-week visits among the different treatment groups. Mean ± Standard error of the mean are displayed.

Treatment	Baseline	6 week	Change†
Scaling and root planing	1.65 ± 0.18	1.15 ± 0.15	0.50 ± 0.24
Laser '50 mJ'	2.05 ± 0.15	1.80 ± 0.17	0.25 ± 0.14
Laser '80 mJ'	1.95 ± 0.15	1.90 ± 0.14	0.05 ± 0.15
Untreated control	1.45 ± 0.15	1.40 ± 0.18	0.05 ± 0.22

† P=0.299, Friedman's test.

Table 3.46 Mean ± standard deviation of pocket depth results for 4 treatment groups (laser '50 mJ', laser '80mJ', scaling, and control). The unit of measurements is mm.

	baseline	6-week	difference
control	6.15 ± 1.87	5.90 ± 1.74	0.25 ± 0.64
laser '50 mJ'	6.35 ± 2.06	5.90 ± 2.07	0.45 ± 1.54
laser '80 mJ'	6.65 ± 1.84	6.40 ± 1.93	0.25 ± 1.19
scaling	5.95 ± 1.43	4.55 ± 1.36†	1.40 ± 1.15‡

† significantly different from baseline, paired t-test $p<0.001$.
‡ Significantly different from all other groups, Duncan's multiple range test ($P<0.05$).

revealed that scaling was significantly superior to all other groups ($p<0.05$).

3.3.3 Total anaerobic counts

The total anaerobic counts of subgingival plaque samples collected immediately after scaling and 80mJ laser treatments showed a significant reduction compared to those collected before treatment ($p<0.01$, paired t-test, Table 3.47). However, after 6 weeks, only the scaling group still showed a significant reduction compared to baseline ($p<0.01$, paired t-test). The differences in counts between the baseline and post-treatment samples and between baseline and 6-week samples were subjected to repeated measures ANOVA and a significant difference was found for the former comparison ($p<0.001$). Duncan's multiple range test elucidated a significant difference between scaling and all other groups ($p<0.05$). However, differences between baseline and 6-week visit samples among the 4 groups were not significantly different ($p=0.059$).

3.3.4 Bleeding on probing

No improvement was observed in bleeding on probing (BOP) for any of the laser treated sites throughout the study (Table 3.48), although the control group showed a little reduction (10%). The scaling group, however experienced a 45% reduction (from 95% to 50%) which proved to be significant ($p=0.0039$, McNemar test).

Table 3.47 Mean ± standard deviation of log CFU at baseline, immediately after treatment and six-week visit for 4 groups (laser '50 mJ', laser '80 mJ', scaling, and control).

	baseline	immediately post-therapy	six week	difference =baseline -post-therapy	difference =baseline - six week
control	4.88 ± 1.62	4.97 ± 1.46	5.31 ± 1.36	-0.09 ± 0.96	-0.43 ± 1.57
laser '50 mJ'	5.54 ± 1.01	5.38 ± 0.69	5.72 ± 0.78	0.16 ± 0.84	-0.18 ±1.12
laser '80 mJ'	5.89 ± 0.90	5.36† ± 0.89	5.93 ± 1.02	0.53 ± 0.71	-0.04 ± 1.00
scaling	5.72 ± 0.93	4.30† ± 1.51	5.09 ± 1.11	1.42‡ ± 1.71	0.63 ± 1.31

† Significantly different from baseline $p<0.01$

‡ Significantly different from all other groups, Duncan's multiple range test, $p<0.05$.

Table 3.48 The number (percentage) of sites with bleeding on probing among 4 groups (laser '50 mJ', laser '80 mJ', scaling, and control).

Treatment	Baseline	6 week	Change
Laser '50 mJ'	19 (95%)	19 (95%)	0 (0%)
Laser '80 mJ'	20 (100%)	20 (100%)	0 (0%)
Control	20 (100%)	18 (90%)	2 (10%)
Scaling	19 (95%)	10 (50%)†	9 (45%)

† Significantly different from baseline, p=0.0039, McNemar test.

3.3.5 Scanning Electron Microscopy (SEM)

SEM examination of laser treated teeth showed no evidence of damage attributable to the laser irradiation. Most tooth surfaces were still covered with substantial pieces of calculus with surface deposits of bacteria, despite laser treatment.

CHAPTER IV

DISCUSSION

4.1 Introduction

Therapeutic devices or drugs with potential periodontal applications may be used either alone or as adjuncts to traditional treatments. However, before it is decided whether the device could be used as an adjunctive treatment, it is usually essential to test the new device or drug alone to evaluate its effect on the biological tissues, potential side effects and to investigate the efficacy of the device *per se*.

Up until now, there has been little research on the laser application in periodontal therapy. Therefore, before investigating or even hypothesising the efficacy of laser as an adjunct to scaling and root planing, it would be essential to examine the effects of the laser energy *per se* on the dental tissues. This approach was followed in this thesis.

On the other hand, considerable research has been done on locally applied antibacterial systems in periodontal therapy. Several systems have been developed and tested *in vitro* and in clinical studies. A number of these systems are now being marketed as adjuncts to scaling and root planing. However, to date, the research on subgingivally applied antibiotics has been mainly focused on the use of these systems as the initial treatment of periodontal disease and little work has been done on the efficacy of these delivery systems for the treatment of sites with an

unfavourable response or recurrence of the disease after traditional mechanical treatment. In this thesis, the effects of subgingival antimicrobial systems as adjunct to scaling and root planing on sites with persistent pocketing were studied.

4.2 The clinical study of locally delivered antimicrobial systems

4.2.1 Clinical response

4.2.1.1 Significant improvement after treatments

All treatment modalities used in this study including scaling and root planing alone, resulted in a significant pocket depth reduction and clinical attachment gain. In addition, other clinical parameters including BOP, suppuration and the MGI scores were significantly improved by all treatments. The results of our investigation support the hypothesis that many sites which do not respond favourably to initial mechanical periodontal therapy, may still benefit from further episodes of scaling and root planing. While these results are in agreement with previous reports by Listgarten *et al.* (1978), they are inconsistent with those of Badersten *et al.* (1984b) who found no difference between a single and 3 courses of scaling and root planing performed every 3 month.

4.2.1.2 Rebound towards baseline

All treatments had improved clinical parameters at the 6 week visit compared to baseline. The healing response was maximised at the 3 month visit. Although the significance of pocket depth reduction and attachment gain were sustained throughout the 6 month period of the study, a rebound towards baseline was observed at the 6 month visit in all treatment groups including scaling and root planing alone. The study sites did not receive any maintenance care throughout the 6 month follow-up period. It could be that the 6 month is too long a period for treated sites to be left without maintenance care. Nyman, Rosling and Lindhe (1975) reported that a programme consisting of professional care every 2 weeks was more effective in arresting attachment loss than one with every 6 months visits.

4.2.1.3 Healing period after root planing alone

The data indicated that the pocket depth and attachment level improvement in the scaling alone group was not complete at the 6 week visit. Although all groups continued to improve between the 6 week and the 3 month visits, this was statistically significant only in the scaling alone group. Therefore, it can be concluded from this study that the optimum time to re-assess the response to scaling and root planing should be longer than 6 weeks after therapy. While Proye *et al.* (1982) reported that the post-therapy attachment gain was complete within 3 weeks,

most reports indicated that after scaling and root planing, gradual improvement in clinical parameters continued to occur up to at least 4 to 5 months post-therapy (Badersten, Nilvéus and Egelberg, 1981; Kaldahl *et al.*, 1988).

4.2.1.4 The effects of locally delivered antimicrobial systems on probing pocket depth, attachment level and recession

Our study revealed that tetracycline fibre therapy as adjunct to scaling and root planing, produced the greatest reduction in pocket depth. At the 6 week visit, the scaling and root planing plus tetracycline fibre was the only treatment group which resulted in a significantly greater pocket depth reduction than that of the scaling and root planing alone group. However, at this time point there were no significant differences among the antimicrobial treatment groups. At the 3 month and 6 month visits no significant differences existed between any of the treatment groups. When 3x the standard deviation of probing pocket depth measurements were used as a threshold to assign a site as one with reduced pocket depth, at the 6 month visit, the root planing plus tetracycline fibre group and the root planing plus metronidazole gel group were found to have resulted in a significantly greater number of sites with reduced pocket depth than the root planing plus minocycline gel group.

The tetracycline fibres have been reported to provide a sustained tetracycline concentration of 1590 μg/ml in periodontal pockets over a 10 day period (Tonetti *et al.*, 1990). Metronidazole gel provided a metronidazole concentration > 1 μg/ml at 50% of sites 24 hours after application, and at 8% of sites 36 hours after application (Stoltze, 1992). Satomi *et al.* (1987) reported a mean GCF concentration of 11.2 μg/ml minocycline 24 hours after application of 2% minocycline gel into pockets. After 48 hours the GCF concentration was 5.4 μg/ml. These reports indicate that variation exists between the substantivity of different drug delivery systems. The results of the present study reflects that local delivery systems with high substantivity may produce the greatest clinical improvement if used as an adjunct to root planing as compared with other systems or mechanical treatment alone.

All treatments resulted in a significant gain in clinical attachment. The scaling plus tetracycline fibre treatment resulted in the greatest improvement in attachment levels. However, the study failed to show significant difference between groups. Nevertheless, at the 6 week visit, scaling plus tetracycline fibre group and scaling plus metronidazole gel group resulted in significantly greater number of sites with attachment gain (as defined by 3x the standard deviation of attachment level measurements) than the scaling and root planing alone group.

All 3 antimicrobial treatments resulted in a greater improvement in pocket depth and attachment level than scaling and root planing alone. This indicates that all 3 antimicrobial treatments offered some beneficial effect over the scaling alone. However, the difference between groups, particularly towards the end of the study, were neither clinically nor statistically important.

Treatments do not heal the periodontal lesions permanently but have to be repeated. The observation that slight rebound towards baseline occurred at the 6 month visit, indicate the interval at which treatment must be repeated, although, due to the risk of antibiotic resistance, too often use of antimicrobial agents is not justified.

The reduction of pocket depth is due to the attachment gain, the formation of long junctional epithelium and the recession. The recession occurs mainly as a result of reduction in inflammatory infiltrate and therefore, shrinkage of tissue. It may also happen as a result of trauma to the gingival tissue as a result of instrumentation. The slight post-treatment attachment loss at shallow sites has been reported in the past and is thought to be in part due to the trauma from instrumentation (Claffey *et al.*, 1988 & 1990). The amount of recession was the greatest in the scaling plus tetracycline fibre group. However, regardless of the absolute amount of the recession and the efficacy of treatments in reducing pocket depth and increasing

attachment level, the proportion of recession to attachment gain was slightly greater in the tetracycline fibre therapy group. In other words, in this group, a greater proportion of pocket depth reduction was due to recession as compared with other groups. This may, in part, be due to some trauma by inevitable tension exerted from the tetracycline fibres to the soft tissue wall of the pocket. However, the clinical importance of this effect, at such a small magnitude, is arguable.

4.2.1.5 Bleeding on probing

It has been demonstrated that the extent of connective tissue inflammation is different between bleeding and non-bleeding sites (Greenstein, *et al.*, 1981; Harper and Robinson 1987; Passo *et al.*, 1988). Despite the reservation of some authors concerning low reproducibility for bleeding on probing scores (Janssen, Faber and van Palenstein Helderman, 1986), evidence suggests that the bleeding on probing decreases after a successful treatment (Caton *et al.*, 1982; Proye *et al.*, 1982) and therefore could be used as part of a diagnostic test to evaluate treatment outcome.

Bleeding on probing scores were reduced significantly at the 6 week visit in all treatment groups. This significant reduction was sustained throughout the whole study period. The reduction in the bleeding on probing was slightly greater in the scaling plus tetracycline fibre

group than other groups. This was consistent with the probing data. However, no statistically significant difference was found between groups at any time point. As with the probing pocket depth and attachment level data, the majority of change took place within the first 6 week period post-therapy.

It has been demonstrated that for a given site, the number of bleeding on probing episodes during a longitudinal monitoring period, is in correlation with the likelihood of attachment loss at that site (Lang *et al.*, 1986 & 1990). Therefore, analysis of sites which show bleeding on probing on 3 consecutive follow-up visits, would be a test with greater positive predictive value than the assessment of bleeding on probing scores for a single visit. In the present study, there were no significant differences between groups in the frequency of sites which showed bleeding on probing on all 3 post-treatment occasions, although a trend was observed whereby the scaling plus tetracycline fibre group showed a lower frequency of such sites compared with the remaining groups.

4.2.1.6 Suppuration

The suppuration decreased dramatically after all treatments at the 6 week visit. This reduction was statistically significant in all but the scaling plus minocycline gel group. However, this lack of statistical significance was most probably due to a lower proportion

of suppurative sites in this group at the baseline, resulting in a greater likelihood of a type II error, and thus, a less powerful statistical test for this group. The suppuration reached zero at the scaling plus tetracycline fibre group at the 6 week visit and it remained zero at the 3 month visit. However, in other groups the scores showed a rebound towards baseline. Notably, in the scaling and root planing alone group a full rebound was observed at this time point. At the 6 month visit, in addition to the scaling alone group, the scaling plus metronidazole gel group also showed an almost full rebound. Interestingly, this was in contrast to the other 2 groups namely the scaling plus tetracycline fibre and the scaling plus minocycline gel groups each having only one suppurating site at this time point.

The rebound in suppuration scores was observed at the 3 month visit, whereas the rebound in other clinical parameters such as probing depth, attachment level, and bleeding on probing was not observed before the 6 month visit. This may indicate that the suppurative sites may be more prone to disease recurrence after the treatment than the nonsuppurative sites.

The notable difference in reduction of suppuration between the root planing alone and the remaining treatments observed at the 3 month visit may indicate that the locally applied antimicrobial systems might have a

particular benefit over the scaling and root planing alone in the treatment of sites which show suppuration despite previous mechanical treatments. The scaling plus tetracycline fibre treatment may be particularly useful in predictably treating such sites. Extremely high concentration of tetracycline over a 10 day period could possibly be effective in suppressing the putative organisms in the pocket. However, care should be taken when applying the tetracycline fibres in sites with a high rate of pus drainage. Tightly inserted fibres in such sites may exacerbate the situation by blocking the drainage route. No such complication occurred in our study. This was presumably due to, as recommended by the manufacturer, deliberately loose placement of fibres at these sites.

4.2.1.7 The Plaque Index, the Modified Gingival Index, and the GCF volumes

The patients' plaque control was generally satisfactory throughout the study, and at all time points the mean PI was lower than 1 for any group. Nevertheless, there was a trend towards a slight increase in the PI in all groups towards the end of the study. It is generally believed that after initial mechanical therapy the PI decreases. However, it should be noted that the subjects in the present study had been previously treated mechanically and their plaque retentive factors had been corrected. Moreover, they all had a satisfactory oral hygiene

awareness and had been practising a high standard of oral hygiene before they were selected for the study. Therefore, the study population was different from an 'untreated' population with low oral hygiene awareness and intact plaque retentive factors and therefore little further improvement in the level of plaque control could be expected from the present study population.

It is generally believed that subjects who participate in a study, may demonstrate an improved oral hygiene, which may result in some improvement on their periodontal disease status even if they have received no active treatment. This, so-called Hawthorne effect, was not evident in our study. It should be noted that the change in the oral hygiene behaviour of patients as a result of the Hawthorne effect could be only a short-term phenomenon as such change would naturally return to normal once the subjects become accustomed to their new treatment. In the present study, the subjects were first seen at a screening visit. It was at this visit when they were first informed of the study. Thus, the Hawthorne effect might have started at the screening visit, and the baseline plaque scores may, therefore, represent an improved PI status compared to the screening visit. However, since no data recording were performed at the screening visit, it was not possible to demonstrate its possible role on the patients' plaque scores. It is possible that once the patients were treated and finished the active phase of the

study, i.e. the treatment phase, they gradually returned back to their routine level of plaque control which was slightly inferior to the baseline scores.

The MGI scores were improved significantly after all treatments and remained significantly different from baseline throughout the entire study period. However, the improvement in MGI in the scaling plus tetracycline fibre treatment group was greater than the other treatment groups. While at the 6 week and the 6 month visits, the improvement in this group was significantly greater than all other groups, there were no significant differences among the remaining groups. However, the difference between groups did not reach the statistically significant level at the 3 month visit.

The advantageous effect of tetracycline fibre therapy in reducing the MGI scores was most likely due to the elimination of pathogenic bacteria by the constant high level of tetracycline in the periodontal pocket. However, the tetracycline family have been reported to have biologic effects other than antibacterial activity on the inflamed tissues, which may ultimately result in the resolution of the inflammation. It has been reported that the tetracyclines could inhibit collagenase activity via binding to metallic ions on matrix metalloproteinase molecules (Golub *et al.*, 1984), inhibit parathyroid hormone-induced bone resorption (Gomes, Golub and

Ramamurthy, 1984), bind to and demineralise the root surface and increase the binding of fibronectin and decrease the binding of laminin (Terranova *et al.*, 1986), and promote fibroblast attachment and spreading on the root surface (Somerman *et al.*, 1988; Rompen *et al.*, 1993). Therefore, the greater resolution of inflammation at sites treated by tetracycline fibres could be in part due to anticollagenolytic activity. Minocycline gel on the other hand might have had a more limited effect than the tetracycline fibres due to lower substantivity of the gel delivery system.

The GCF volume reduction at the 6 week visit was significant only at sites treated by scaling and root planing plus tetracycline fibres. The GCF volumes at subsequent follow-up time points were not significantly different from baseline at any time point. This could be a reflection of rebound to pre-treatment values as well as sampling error and biological variation.

4.2.2 Methodological considerations

4.2.2.1 Screening of the sites

In the present study the selection of sites was based on cross-sectional screening. At the screening visit 4 sites with probing depth ≥ 5 mm and bleeding on probing despite previous treatments were selected. One might question the method of screening in this study as to whether this method was able to pick up the disease active sites. Some

authors have suggested that the inclusion of sites in a longitudinal study of therapeutic modalities for periodontitis should be based on the longitudinal screening of sites, and that only those sites which would show an attachment loss exceeding a predetermined threshold over the screening period should be selected for the study (Page and De Rouen, 1992). In reply to this question it should be noted that firstly, a study with longitudinal screening would require much more time and manpower than a study with cross sectional screening such as the present study (Goodson, 1992). In addition, a pragmatic consideration is that in practice, antimicrobials would be used on sites which appeared resistant to treatment based on residual pocketing and BOP.

Furthermore, if the concept of disease activity and the random burst model is true, some active sites could be selected by longitudinal screening over a period. However, there would not be any certainty as to whether all of the selected sites would have continued to remain active. The activity of some of them could have been limited to a short time coinciding with the screening period, followed by a period of quiescence before the end of screening period, resulting in the inclusion of some false positives in the study. Therefore, if the concept of disease activity is accepted, even by monitoring sites

for disease activity before site selection, the study would not be immune from inclusion of quiescent sites.

Finally, the true distribution and model for periodontal disease progression is as yet unknown and the effort of clinical scientists and statisticians to disclose the true model is hampered by the large measurement errors of the available diagnostic tools. Until more advanced technology allows for the error free detection of attachment level changes over time, the true model will remain unknown (Sterne, 1988). In fact when the most reproducible technique using an automated electronic probe was used, the regression analysis revealed that 76% of the sites showed attachment loss which is consistent with a continuous model and only a small subset of sites showed either bursts of activity or remission of the disease (Jeffcoat and Reddy, 1991b).

4.2.2.2 Patient population

The subjects of the present study were selected among patients which responded poorly to previous mechanical treatments or showed recurrence of the disease after transient improvement. Although a significant improvement was achieved as a result of scaling alone, this was lower than would be expected if carried out as the initial treatment on an 'untreated' periodontitis population. Usually, following the initial treatment, a marked pocket reduction and shrinkage occurs due to the elimination of

subgingival plaque, calculus and other plaque retentive factors. Therefore, somewhat less improvement could be expected from the scaling and root planing alone if some of these factors have already been reduced by initial therapy.

In practice, it is not feasible to use antibiotics as the initial treatment of untreated periodontal disease. In fact, the only justified use of chemical treatment in periodontal therapy is the treatment of sites with persistent disease despite previous mechanical treatment.

4.2.2.3 Parallel design

This was a parallel-designed study. Such a design is more likely to disclose true differences between treatment modalities which may be associated with a considerable-carry-over effect. If a split mouth design was used, the wash-out of antimicrobial agents through saliva could affect the microbial flora in study sites which received other treatments. This, in addition to boosting the systemic response due to removal of antigenic challenge or the inoculation effect following mechanical instrumentation, could mask true differences between groups if a split mouth design was used (Imrey, 1986).

4.2.2.4 Sample size estimation

Estimation of sample size was carried out in a pilot study with the same measurement techniques. Nine patients were treated, each by scaling and root planing plus one of the 3 local delivery antimicrobial systems. It was estimated that 13 patients in each group would provide enough statistical power (with a probability of 80%) to disclose a significant difference ($p<0.05$) in the 6-week pocket depth reduction between the scaling and tetracycline fibre therapy and the remaining treatments. The assumption was that the standard deviation and the mean difference of pocket depth reductions would be exactly repeated in the main study. However, the final analysis revealed that the difference between the scaling plus tetracycline fibre and the scaling plus metronidazole gel or minocycline gel groups did not reach the statistical significance. However, the difference between the scaling and root planing plus tetracycline fibre group, and the scaling and root planing alone proved to be significant at the 6 week visit. The mean difference between treatments was greater in the pilot study than it was in the final analysis of the full study. This inconsistency could be attributed to the small number of subjects used in the pilot study. A parallel design was selected for the study to prevent the so-called carry-over effects between treatments. However, by using this design the variation among individuals in terms of treatment response could have had a greater role than if a split mouth design was used, especially when the

number of subjects were too small in the pilot study. Therefore, while a pilot study could provide a rough estimation of approximate sample size, it could not be entirely reflective of the final study results.

4.2.2.5 Measurements of attachment level and pocket depth

In the present study, an electronic pressure sensitive probe was used to measure attachment levels and probing pocket depth. The measurements were taken in duplicate and a single examiner was used for the measurements throughout the study. The reliability of clinical measurements is often expressed as the standard deviation of differences between repeated measurements. In this study, the standard deviation of attachment levels using the Stent Florida Probe was 0.60 mm. When duplicate measurements were used instead of single ones, the standard deviation reached 0.34 mm for attachment levels and 0.42 mm for pocket depth measurements. The overall small measurement error obtained in this study might also have been due, in part, to the position of sites in this study, the majority of which were the anterior flat surfaces. It has been reported that anterior sites are measured more reproducibly than molar flat surfaces or molar furcation sites (Loos, Kiger and Egelberg, 1987).

The reproducibility of attachment level recordings was greater than that of pocket depth recordings as indicated

by a smaller standard deviation for the former. Moreover, the results indicated that the standard error of the mean of attachment gain was consistently smaller than that of the pocket depth reduction across the treatment groups and at any time point. The greater reproducibility of the attachment level measurements over the pocket depth measurements could be attributed to the use of the acrylic stents with reference points (grooves) for attachment level measurements which served as reference points both at the apico-coronal, and the mesio-distal directions. On the other hand, the pocket depth measurements relied on the placement of the probe tip as close as possible to the contact point by the examiner, a factor which did not have a role in the attachment level measurements. Moreover, the vertical rest seat for the edge of the probe sleeve differed for the pocket depth and attachment level Florida Probes. While the acrylic stent provided a rather firm seat for the attachment handpiece, the gingival margin was a more resilient seat for the pocket depth handpiece sleeve. Previous report on the reproducibility of the Florida probes (Gibbs *et al.* 1988) is in agreement with our finding of slightly higher reproducibility of the attachment level over pocket depth measurements.

4.2.3 The effect of baseline pocket depth on the response to treatment

Many investigators have postulated that sites with an initially greater pocket depth may show a greater pocket

depth reduction and attachment gain following non-surgical therapy (Badersten *et al.*, 1984b; Badersten, Nilvéus and Egelberg, 1987; Claffey *et al.*, 1988 & 1990; Claffey, 1991). This phenomenon was also clearly demonstrated in the present study. More pocket depth reduction in deeper sites is to be expected, as the shrinkage (and thus pocket depth reduction) is in proportion to the initial bulk of inflamed tissue. More attachment gain in deeper sites may indicate that the amount of re-adaptation of tissues at the apical aspect of the pocket may be related to the height of the soft tissues initially. Deeper sites may heal with a greater amount of adaptation of the pocket lining to the root surface or with a less penetrable junctional epithelium (Egelberg and Claffey, 1994). The analysis of pocket depth or attachment gain data should therefore, take into account this important variation in treatment response among the study sites. In the present study, the General Linear Model analysis was used, which allowed simultaneous assessment of the significance of baseline probing pocket depth effect on the pocket depth reduction (or attachment gain) together with the assessment of the significance of the main effect i.e., the treatment type. This analysis revealed that the baseline pocket depth had a strong and often significant effect on the pocket depth reduction and attachment gain. Moreover, this effect was often stronger than the effect of treatment type. The analysis consistently revealed that there was no significant interaction between the

effect of baseline pocket depth and the effect of treatment type. This indicates that the effect of having deeper baseline pocket depth resulting in a greater improvement, did not depend on the treatment type. This lack of interaction is a prerequisite for validly interpreting the effect of individual terms in the analysis.

Finally, it has been reported that a small proportion of the effect of initial pocket depth on the magnitude of pocket depth reduction and attachment gain may be due to a statistical artefact known as 'regression towards the mean' (Blomqvist, 1987; Egelberg, 1989). Due to this phenomenon, the measurement error causes some sites to be recorded deeper (or shallower) than their real depth at baseline. Therefore, even if no treatment is performed, in the next data recordings, the majority of such sites would probably be recorded closer to their actual depth. Thus, in the absence of any real change, this would falsely indicate that the site has become shallower (or deeper). The greater the measurement error, the greater would be the 'regression towards the mean' (Blomqvist, 1987). In the present study, while the regression towards the mean might have played a role, its effect must have been reduced by the use of electronic pressure sensitive probes and duplicate measurements.

4.2.4 The effect of smoking

During the past few years, there has been an explosion of evidence concerning the effects of smoking on periodontal tissues. It has been found that smokers are more susceptible to periodontal disease than non-smokers (Ismail, Burt and Eklund, 1983; Linden and Mullally, 1994). A significant association exists between the current smoking status and advanced periodontitis and bone loss (Bergström, Eliasson and Preber, 1991; Horning, Hatch and Cohen, 1992; Locker and Leake 1993). MacFarlane *et al.* (1992) reported that 90% of patients responding poorly to periodontal treatment were smokers. Stoltenberg *et al.* (1993) reported that cigarette smoking is a stronger risk indicator than any of the putative periodontal pathogenic micro-organisms. Preber and Bergström (1986 & 1990) and Ah *et al.* (1994) reported that smokers present a less favourable response to surgical and non-surgical periodontal treatment. Tonetti, Pini-Prato and Cortellini (1995) reported a significantly less attachment gain following guided tissue regeneration procedures in smoker as compared with non-smoker subjects. Moreover, they reported that smoking was a significant risk marker for treatment failure at 1 year follow-up after an initial success.

The results of the present study are in agreement with previous studies which found an important role for cigarette smoking in periodontal disease. Regardless of

the treatment type, the pocket depth reduction and attachment gain were consistently greater among non-smokers as compared with smokers. When the effects of smoking status together with the baseline pocket depth on the treatment outcome were assessed using a multivariate analysis (GLM), the effect of smoking was found to be significant at the 3 month and 6 month pocket depth reductions. Moreover, the effect of smoking on attachment gain was found to be significant at all 3 follow-up occasions. These findings are in agreement with previous reports (quoted earlier) on the role of smoking in periodontal disease.

An important consistent finding was the presence of a significant interaction between the smoking effect and baseline pocket depth effect at all follow-up visits for both pocket depth reduction and attachment gain. This indicates that the inhibitory effect of smoking on treatment response is more pronounced at initially deeper sites. These findings are in agreement with those reported recently by Ah *et al.* (1994) who found that the unfavourable response in smoking subjects was more pronounced and significant at the initially deeper sites. These findings may have important prognostic implications in the treatment of periodontal disease. This phenomenon might be related to the reportedly impaired function of fibroblasts in smokers (Raulin *et al.*, 1988). Fibroblasts have been shown to bind and internalise nicotine (Hanes,

Schuster and Lubas, 1991). The clinical attachment gain observed after non-surgical periodontal therapy is mainly due to an increase in density of the connective tissue fibres. A deep pocket represents a pocket with a soft tissue wall which is less resistant to probe penetration due to a less dense collagen component in the connective tissue. After treatment and elimination of inflammation, in a non-smoking subject with a presumably normal fibroblast function, the density of functional collagen fibres will be largely recovered and the amount of expected attachment gain is proportional to the initial degree of inflammation i.e., the initial degree of probe-penetrability of the tissues as well as the initial bulk of soft tissue wall of the pocket which contained disintegrated connective tissue fibres. In other words, the deeper the initial pocket depth, the greater the post-treatment attachment gain (and thus, pocket depth reduction). On the other hand, in a subject with a presumably impaired fibroblast function, the amount of tissue adaptation is reduced as a result of fibroblasts failing to increase the density of collagen fibres. The data of the present study suggests that in smoking subjects the amount of tissue adaptation after therapy: a) is lower than seen in non-smoking subjects; and b) has a weak relationship with the initial degree of tissue resistance to probe. The mechanisms of healing among the smokers may, therefore, be somewhat different from the non-smokers. Other important biological effects of

nicotine on the periodontium includes decreased phagocytosis, chemotaxis and viability of neutrophils from oral tissues (Kenney *et al.*, 1977; Kraal *et al.*, 1977), and impaired production of IgA, IgG, and IgM (Holt, 1987; Johnson *et al.*, 1990) which all could be potentially important factors in protecting the host tissues from re-infection during the healing process.

Our data also indicated that the rebound in pocket depth reduction and attachment gain observed at the 6 month visit, was, on average, entirely limited to the smoking subjects. This is in close agreement with MacFarlane *et al.* (1992) who reported a prevalence of 90% smokers among refractory periodontitis patients.

It has been reported that smokers generally have a poorer oral hygiene (Ismail et al., 1983; Ah et al., 1994) and thus, it has been speculated that this difference in plaque level might be responsible, in part, for less favourable treatment response in the smoker subjects. In the present study, most subjects were highly motivated and had little plaque retentive factors. The plaque scores in non-smokers were not lower than those of smokers. This allowed for a comparison between 2 categories matched for their plaque levels. Therefore, the less favourable healing in smokers could not be attributable to the plaque control level. This is in agreement with the studies of Ismail *et al.* (1983), and Tonetti *et al.* (1995) who after

controlling for plaque levels, still found a higher periodontal disease score or a less favourable response to periodontal therapy among the smokers.

Nicotine may cause a vasoconstriction in the peripheral blood vessels (Kardachi and Clarke, 1974; Clarke, Shephard and Hirsch, 1981; Shields, 1977). In our study, one of the criteria for site selection was the presence of bleeding on probing, thus it was not possible to assess the difference in baseline bleeding tendency between smokers and non-smokers. However, the results indicated that the baseline GCF volumes were significantly lower among smokers than non-smokers. After treatments, the decrease in the GCF volume of smokers was less than non-smokers. However, their actual mean GCF volumes still remained lower than non-smokers. These findings are consistent with both a diminished peripheral blood flow (leading to a diminished GCF flow) and a reduced response to periodontal therapy among the smoker subjects.

One limitation of the present study is that the number of cigarettes smoked per day was not taken into consideration. While this would have provided a stronger and more conclusive analysis, it might also have necessitated the inclusion of a greater number of subjects due to the presence of a greater number of cells in the multivariate analysis.

4.2.5 The effect of previous surgery

It was observed that the sites which had been treated by means of periodontal flap surgery, generally showed less improvement after treatments as compared with those which had not been treated surgically. Sites with previous surgery may have greater recession, so smaller pocket depth reduction due to this element is likely.

4.2.6 Safety

No serious adverse effect was observed as a result of treatment with locally applied antimicrobial systems. This could be a potentially important advantage over the systemic usage of antibiotics. Systemic antibiotic therapy is occasionally associated with side effects (see chapter 1) which may complicate the decision making as to when to prescribe a systemic antibiotic for a chronic and non-fatal disease such as periodontitis. Nevertheless, the chance of occurrence of antibiotic resistant bacteria at the site of treatment should not be entirely ruled out if local delivery systems are used. Metronidazole antibiotic resistance is not common. It has been reported that the occurrence of minocycline resistant bacteria after minocycline gel therapy is not more than that observed following the systemic use of minocycline (Preus *et al.*, 1995). Goodson and Tanner (1992) reported that the increase in the proportion of tetracycline resistant bacteria after tetracycline fibre therapy was mainly due to an increase in the proportion of Gram positive coccal

micro-organisms which were assumed to be associated with periodontal health rather than disease. They also reported that this increase in tetracycline resistance was not associated with increase in penicillin resistance, and therefore, they considered the risk of development of plasmid mediated tetracycline resistance low. While, it appears that the use of local antimicrobial systems may involve less risk for patients, further research on this area is indicated due to increase in the use of local antimicrobial systems. It should also be determined how often antimicrobial systems could be used without taking the risk of inducing a non-responsive antibiotic resistant micro-flora, if these systems are going to be used as adjunctive tools in maintenance therapy.

4.2.7 Treatment time and cost-effectiveness

Tetracycline fibre therapy was quite time-consuming. It took, on average, 8.2 minute to treat each pocket by tetracycline fibres. Considering the time needed to apply the adhesive and periodontal dressing, normally a one-hour appointment was required to complete the tetracycline fibre therapy for 4 sites. Moreover, an additional visit was required to remove the fibres. On the other hand, although gel therapy *per se* did not take much time, minocycline gel therapy required 3 separate visits, each 2 weeks apart, and metronidazole gel therapy required 2 visits with a one week interval. These complicated treatment disciplines may affect the cost of the treatment

by dental practitioners. In this way, it may also affect the patient acceptance of the treatments. Together with the rather expensive price of the systems, these considerations should be taken into account when one is weighing the potential benefits of the local antimicrobial systems over the traditional treatment strategies.

4.3 The *in vitro* laser study

Increasing the energy setting and duration of the laser, generally resulted in increase in the severity of damage to calculus, cementum and dentine, although it was observed that cementum and dentine were not affected using low energy settings and short durations. On the other hand, calculus was more or less affected using all energy settings and durations. It seems that regardless of the energy and duration of the laser irradiation, there were more severe physical changes to calculus compared to either dentine or cementum. One of the reasons may be that subgingival calculus is usually darker than the dental tissues, and therefore absorbs more laser energy than the other tissues. The wavelength of the Nd:YAG laser beam (1064nm) is not in the visible spectrum, but it is quite near the visible spectrum. Visible light is highly absorbed to the subgingival calculus and thus, the laser wavelength would also be expected to be highly absorbed by the subgingival calculus. Even with very low energy and duration (50mJ, 10pps, 1sec), the laser could

cause some physical changes to calculus, whereas it did not affect any of the cementum specimens using 50mJ, 10pps for either 1 or 5 sec. On the other hand, using 100mJ, 20pps for 5 sec the laser caused severe destruction of calculus, dentine and cementum.

Interestingly, when the laser energy was strong enough and calculus was thin enough to be evaporated completely, some parts of the underlying dental tissues were affected too. It seems that none of the laser settings could differentiate calculus and dental tissues and all were affected (e.g. Fig. 3.11). Perhaps calculus causes more laser power absorption to the area, thus more heat is generated. This heat may affect not only the calculus itself but also the adjacent tissues. SEM showed that there were no distinct boundaries between cementum and calculus on the internal wall of the laser lesions. No specimen was found in which the laser could evaporate calculus completely without evaporating cementum. This inability to differentiate foreign and host material may limit the clinical application of the laser for calculus removal in periodontal pockets.

Results showed that the value of power setting, number of pulses per second and radiation duration have an additive contribution to the damage score. However, the study has low power to distinguish between different models of the effect of power setting, number of pulses per second and

length of time on the mean damage score in view of the small overall sample size and the arbitrary nature of the mean damage score.

This study has attempted to quantify the effects caused by the laser on the root surface. In previous studies, the laser handpiece was moved on the root surfaces with an almost parallel angulation to the root surface, where the whole surface was exposed using an overlapping movement of the laser tip. As stated previously it may be possible that some points on the root surface had multiple treatments because of the overlapping movement. On the other hand, viewing the small size of optic fibre diameter in relation to the whole treatment area, there was a possibility of undertreating some parts. It was not clear, therefore, which part of the treated surface could be attributed to the particular laser settings. In addition, the degree of damage might have been influenced partly by the speed of movements, a factor which was not controlled in previous studies. To reduce the methodological variability, it was decided to lase only one point for a specific period of time without moving the fibre. This protocol appears to have been highly successful.

Results have indicated that specific dentine and cementum specimens appear to have different degrees of susceptibility to the laser. The variability of this

susceptibility was evidenced by the two following extremes, one of the dentine specimens was not affected by any of the eight laser settings, whereas another was more or less affected by all 8 settings. Others fell somewhere between the two. This phenomenon may be the result of variability in colour, texture, percentage of water content, or some other inherent host or site factors which may influence the degree of energy absorption. Such unpredictable variation may limit the clinical application of lasers in periodontal pockets. Moreover, in most teeth, different sites of the same calculus specimen showed different susceptibility to laser exposure. Out of 32 teeth only 11 teeth showed equal damage scores for all treated sites. This is probably due to variation in the colour, thickness, composition, and other characteristics of calculus in the same tooth.

To date there is no study on the effect of Nd:YAG lasers on the periodontal ligament. Trylovich *et al.* (1992) reported a decreased compatibility of fibroblasts on the laser treated root surface. Moreover, in a study with cultured human skin fibroblasts, Abergel *et al.* (1984) reported collagen production and DNA synthesis to be delayed by Nd:YAG laser exposure. It is likely that periodontal fibroblasts show similar response after laser exposure.

Further work is necessary to clarify root tissue behaviour to the laser irradiation. In conclusion, the laser can cause physical changes on calculus under laboratory conditions, even if used with low energies and short durations. However, calculus free dental tissues were not affected by low energies of laser (50mJ-10pps-1sec). In addition, there was variability in the degree of susceptibility of different teeth and different parts of the same tooth to the laser.

4.4 The clinical laser study

Most treatment modalities used in periodontal therapy aim to reduce the bacterial plaque on the root surface and periodontal tissues to levels compatible with the host response such that the disease will be controlled. The effectiveness of scaling and root planing in the treatment of periodontal disease is universally accepted (Axelsson and Lindhe, 1981; Kaldahl *et al.*, 1988; Morrison, Ramfjord and Hill, 1980; Badersten *et al.*, 1984b). In theory, the ability of laser energy to kill and ablate bacteria might be useful in eliminating bacterial plaque and thereby reducing probing depth and bleeding on probing. The purpose of the present study was to determine whether the relatively low level laser pulse energies were sufficient to reduce microbial numbers, and improve the clinical parameters of periodontal disease and to determine whether

these energy levels had any detrimental effect on the root surface *in vivo*.

All groups (including the untreated control) showed some reduction in pocket depth at the re-assessment visit compared to baseline. The slight reduction in pocket depth in all groups may be explained by the improved oral hygiene of the patients. Three weeks before baseline, they received a full oral hygiene instruction. Furthermore, after baseline, there was still a slight improvement in the plaque scores for all groups, however, supragingival plaque control has been shown to have a minor effect on pocket depth (Beltrami, Bickel and Baehni, 1987).

The patients had a relatively low gingival index at the baseline (mean= 1.77) and the scaling group showed the highest reduction in gingival index (0.5) and bleeding on probing, whereas the reduction in the gingival index of the other groups was minimal and could be attributed to improved oral hygiene.

While the gingival index may reflect the presence of inflammation at the periphery of the periodontium, the absence of bleeding on probing may provide a better assessment of health for the whole periodontium. Only the scaling group showed a significant reduction in the incidence of bleeding on probing, suggesting that the

laser treatments used in this study were not capable of reducing the level of inflammation within the pockets.

The post-therapy reduction in anaerobic counts in the scaling group was significantly greater than all other groups; 80mJ laser treatment produced only a 3 fold reduction in bacterial numbers which failed to change significantly the clinical parameters during the course of the study.

Tseng *et al.* (1991b) in an SEM study, reported that very few periodontopathogenic microorganisms were observed on contaminated root segments when lased at 1.5W and 10pps for one minute using a Nd:YAG laser *in vitro*. Cobb *et al.* (1992) in an *in vivo* study evaluated the antimicrobial effect of the Nd:YAG laser. Laser treatment was performed at 1.75W, 2.25W and 3.00W for 1 and 3 minutes either before or after hand instrumentation while some teeth were treated using the laser alone. They reported a significant decrease in the proportion of *A.a.*, *P.g.*, and *P.i.* in samples collected pre and post treatment. When two teeth were sampled seven days after laser treatment, one showed an increase in the numbers of recovered *P.i.* that was credited to recolonisation of bacteria in laser-induced porosities of the root surface. In this study, lower pulse energies than those used by Tseng *et al.* (1991b) and Cobb *et al.* (1992) were used. This was done to limit any thermal effect which laser treatment may have

on the cementum and to test whether 80mJ pulses (3 mins exposure) which yielded 99.9% kills for some species (Whitters *et al.*, 1994) were as successful in inhibiting bacteria *in vivo*. In previous studies (Tseng *et al.*, 1991b; Cobb *et al.*, 1992; Whitters *et al.*, 1994), different methods and microbial volumes have been investigated making determination of the energy dose per microbial cell difficult. This also makes determination of the optimal laser parameter for bacterial killing difficult.

SEM examination of the laser treated surfaces in this investigation revealed no sign of physical damage due to laser energy. This observation is contrary to that reported by Trylovich *et al.* (1992) who used an energy setting of 80mJ and 10pps for 1 minute *in vitro* and observed by SEM physical damage on the root surface. This difference may be due to lasing technique since the handpiece was held perpendicular to the root surface by Trylovich *et al.* but almost parallel to the surface in the present study. The laser energy delivered to the root surface is higher if the fibre is held perpendicular to the root surface. Moreover, the presence of biological fluid in the pocket could have a cooling effect on the root surface and might prevent root damage. The root surface adjacent to the pocket is located subgingivally and in order for the laser energy to have access to the

surface, the handpiece has to be kept almost parallel to the surface.

In the present study, teeth with a poor prognosis were used because there was no certainty as to whether the laser energy would have a permanent detrimental effect on the root surface, pulp or periodontium. The Nd:YAG laser energy has a wavelength of 1064nm and is capable of penetrating through tissues into a considerable depth before its energy becomes attenuated.

Trylovich *et al.* (1992) evaluated the biocompatibility of root surfaces treated by pulsed Nd:YAG laser at energy levels of 80mJ, 10pps and higher and reported a decreased degree of fibroblast attachment on the root segments following the laser treatment. Similar findings were reported by Thomas *et al.* (1994). They also observed that subsequent root planing of the laser treated root reversed only some of the impaired surface biocompatibility of the root. Spencer *et al.* (1992) characterized the chemical structure of lased root surfaces using Fourier transform infrared photoacoustic spectroscopy. They reported a decrease in the protein/mineral ratio on the cementum surfaces lased at energy level of 80mJ and 10pps and suggested that the ammonium ion content was increased as a result of protein denaturation. Clearly, further research is required to determine a clinically effective and yet

safe method for applying laser energy in the treatment of periodontally diseased tissues.

4.5 Conclusion and further studies

Of the therapeutic systems investigated in this thesis, subgingivally applied antimicrobial systems appeared to be potentially useful adjuncts to scaling and root planing. Laser therapy, on the other hand, did not appear to be a beneficial and safe alternative to scaling and root planing. The clinical study of locally delivered antimicrobial systems revealed that the use of these systems as adjunct to scaling and root planing may offer some benefit over the scaling and root planing alone at sites which appeared to have an unfavourable response to previous mechanical therapy. Scaling plus tetracycline fibre offered the greatest advantage especially in the treatment of suppurative sites. It is recommended to use these systems as adjunct to scaling and root planing. Furthermore, since the scaling and root planing either with or without surgical access is often quite effective in arresting untreated periodontal lesions, and in view of the tetracycline fibre insertion being time consuming, it is recommended to use these systems only for selected sites i.e. sites with a poor response to initial therapy. In this study, tetracycline fibre, a system with a non-resorbable vehicle produced a greater improvement than the other 2 systems with resorbable vehicles. Further research could possibly focus on the development of bioresorbable

vehicles with improved substantivity. Furthermore, if longer-lasting resorbable vehicles can be developed, the need for repeated application of the systems will be eliminated.

The results of studies on Nd:YAG laser revealed that when ablating subgingival calculus using the Nd:YAG laser, the cementum and dentine are damaged. Moreover, there is a large variability on the absorption of laser energy on calculus or dental hard tissues. This makes the Nd:YAG laser an instrument with unpredictable outcome. When the Nd:YAG laser energy was used at lower energy levels in a clinical study, although no physical damage and melting down was observed on the root surface, it did not result in a significant bacterial kill or improvement of clinical parameters as compared with scaling and root planing. Our conclusion is that the Nd:YAG laser has little application in the treatment of plaque associated periodontal disease. Before introducing lasers into routine periodontal therapy, further research should be directed towards the development of laser energy wavelengths with selective absorption for calculus and subgingival bacteria and little absorption for cementum, dentine and soft tissues.

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List of publications:

The following papers are directly related to the work presented in this thesis:

Radvar, M., Creanor, S.L., Gilmour, W.H., Payne, A.P., McGadey, J., Foye, R.H., Whitters, C.J. & Kinane, D.F. (1995) An evaluation of the effects of an Nd:YAG laser on subgingival calculus, dentine and cementum. An in vitro study. *Journal of Clinical Periodontology,* **22,** 71-77.

Radvar, M., MacFarlane, T.W., MacKenzie, D., Whitters, C.J., Payne, A.P. & Kinane, D.F. (1996) An evaluation of the Nd:YAG laser in periodontal pocket therapy. *British Dental Journal,* (In press).

Radvar, M., Pourtaghi, N. & Kinane, D.F. A comparative evaluation of 3 locally delivered antimicrobial systems used in conjunction with scaling and root planing in persistent periodontal pockets. (submitted to the *Journal of Periodontology*).

Radvar, M., Pourtaghi, N. & Kinane, D.F. The effect of cigarette smoking on the outcome of antimicrobial and mechanical periodontal therapy. (submitted to the *Journal of Periodontology).*

The following paper is indirectly related to the work presented in this thesis:

Pourtaghi, N., Radvar, M., Mooney, J. & Kinane, D.F. The effect of subgingival antimicrobial therapy on the levels of stromelysin and tissue inhibitor of metalloproteinases in gingival crevicular fluid. (submitted to the *Journal of Periodontology).*

Abstract presentations:

Radvar, M., Creanor, S.L., Gilmour, W.H., Payne, A.P., McGadey, J., Foye, R.H., Whitters, C.J. & Kinane, D.F. (1994) The effects of an Nd:YAG laser on subgingival calculus, dentine and cementum. *Journal of Dental Research,* **73,** 805, abstract 145.

Radvar, M., MacFarlane, T.W., MacKenzie, D., Whitters, C.J., Payne, A.P. & Kinane, D.F. (1995) An evaluation of

the Nd:YAG laser in periodontal pocket therapy. *Journal of Dental Research,* **74,** 852, abstract 241.

Radvar, M., Pourtaghi, N. & Kinane, D.F. (1996) Smoking plays an important role in the outcome of periodontal treatment. *Journal of Dental Research,* (in press).

Kinane, D.F., Radvar, M. & Pourtaghi, N. (1996) A 6-month follow-up evaluation of 3 locally delivered antimicrobial systems as adjuncts to scaling and root planing in persistent periodontal pockets. *Journal of Dental Research,* (in press).

Pourtaghi, N., Radvar, M. & Kinane, D.F. (1996) Matrix metalloproteinases levels after subgingival antimicrobial therapy. *Journal of Dental Research,* (in press).

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An evaluation of the effects of an Nd:YAG laser on subgingival calculus, dentine and cementum

An in vitro study

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Radvar M, Creanor SL, Gilmour WH, Payne AP, McGadey J, Foye RH, Whitters CJ, Kinane DF: An evaluation of the effects of an Nd:YAG laser on subgingival calculus, dentine and cementum. An in vitro study. J Clin Periodontol 1995; 22: 71–77. © Munksgaard, 1995.

Abstract. The aim of this study was to evaluate the effects of Nd:YAG laser treatment on subgingival calculus, cementum and dentine, *in vitro* at different power settings and durations. The study included 2 experiments. In the 1st experiment, 32 extracted teeth with calculus were divided into 8 laser treatment groups. Each tooth was treated on 2, 3 or 4 sites. In the 2nd experiment, 3 extracted cementum covered teeth and 3 extracted root planed teeth with exposed dentine were selected. 1 surface of each tooth was subjected to 8 different laser treatments. In both experiments, all specimens were assessed using scanning electron microscopy. Micrographs were taken from each treated site at ×100 and ×750 magnifications. An arbitrary scale (from 0 to 3) was used to score the degree of damage caused by the laser. Generally, the laser caused greater damage on calculus than either cementum or dentine. Linear regression analysis showed that higher total energy input caused a greater mean damage score on calculus (R^2= 66%, $p<0.001$). 3-way analysis of variance showed that for calculus, the power setting, number of pulses per second and the duration of exposure contributed independently to the mean damage score in an additive way. Cementum specimens were not affected by treatment 1 (50 mJ, 10 pps, 1 s), treatment 2 (50 mJ, 10 pps, 5 s), and treatment 5 (50 mJ, 20 pps, 1 s). Dentine specimens were not affected by treatment 1 (50 mJ, 10 pps, 1 s). The results also showed that there was variability in susceptibility of different teeth and different parts of each tooth which was true for calculus, cementum and dentine.

Key words: Nd:YAG laser; subgingival calculus; dentine; cementum; SEM

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Certain manufacturers and researchers have suggested that the Nd:YAG laser could be used to facilitate root planing (American Dental Laser Company, product manual, Tseng et al. 1991a). Some studies have reported that the laser could potentially reduce the number of curette strokes necessary for removing calculus (Tseng et al. 1991a). The laser might also reduce the sub-gingival bacteiral flora in vitro (Tseng et al. 1991b, White et al. 1991). Cobb et al. (1992) have examined the bactericidal effect of Nd:YAG laser in vivo, and concluded that laser exposure of the root surface can significantly decrease the number of periodontal pathogens.

Morlock et al. (1992) examined laser effects using SEM and described the effects caused by the laser energy on the root surface in vitro. Trylovich et al. (1992) reported that the laser decreased the biocompatiblity of the root surface for fibroblast attachment. Using Fourier transform photo acoustic infra-red spectroscopy, Spencer et al. (1992) showed that the laser reduced the protein/mineral ratio and induced chemical changes on the root surface.

Previous studies have used overlapping movements in an attempt to mimic the clinical therapeutic use, the laser hand piece tip being kept in contact with the root surface, with an almost parallel angulation to the surface. Overlapping movements attempt to ensure that the whole surface is treated. It is possible, however, that some areas were over treated by this overlapping technique. On the other hand, viewing the small diameter of the laser handpiece (320 μm), some areas might not have been treated at all. Moreover, there was no control on the speed of the move-

ments. To do a comparative evaluation of laser effects on different tissues at different power settings and durations, it is necessary to standardize the experimental method. In this study a laser beam held in a fixed position, perpendicular to the surface has been used. This study aimed to examine the effect of laser on calculus and root tissues. A further objective was to determine if there was any difference in the susceptibility of calculus, cementum, and dentine to laser exposure. This study included two separate experiments the results of which were compared and discussed together.

Material and Methods

Experimental design and specimen preparation

First experiment, calculus

32 freshly extracted teeth with subgingival calculus were selected. Using tweezers, remaining tags of periodontal ligament were removed and this was confirmed using a light microscope (×10 magnification). The teeth were kept in %0.12 thymol solution until the time of the experiment. Calculus covered teeth were randomly divided into 8 different treatment groups of 4. The laser treatment was carried out using an Nd:YAG laser (American Dental Laser, model d.lase-300, Sunrise Technology Inc., Fremont, California). Each group were subjected to the laser treatment using predetermined settings and durations. Laser settings included: 50 mJ–10 pps (pulse/s), 50 mJ–20 pps, 100 mJ–10 pps, 100 mJ–20 pps. Each setting was used for 1 and 5 s, thus providing 8 various settings and durations. Depending on the extent of calculus, each tooth was treated on either 2, 3 or 4 parts of the calculus using the same power setting and duration. During the experiment, the laser handpiece was held perpendicularly in contact with the calculus surface using a calibrated jig to prevent movement.

Fig. 1. Unlased calculus (original magnification ×750; bar=10 μm).

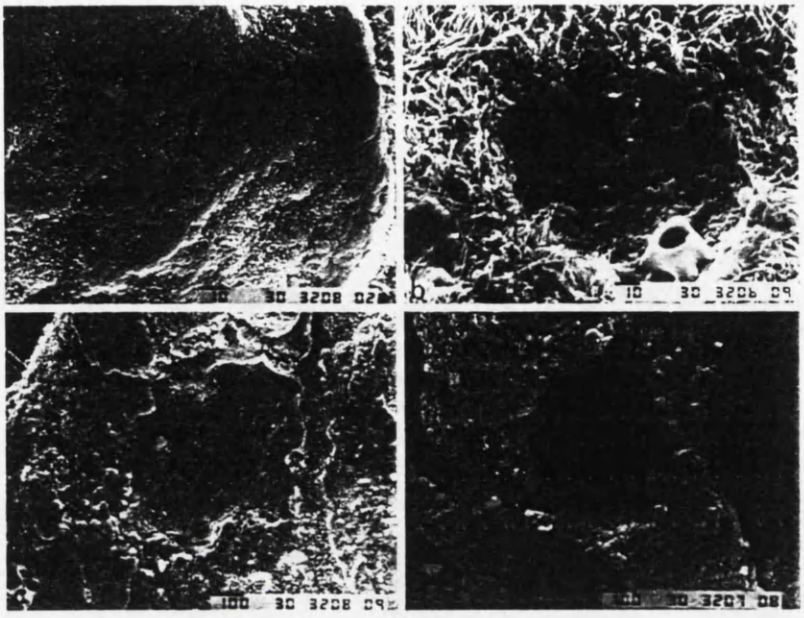

Fig. 2. (a) Lased calculus (using 50 mJ, 20 pps, 1 s) "score 0". There is no laser induced change (original magnification, ×750; bar=10 μm). (b) Lased calculus (using 50 mJ, 10 pps, 1 s) "score 1". Superficial bacteria have been destroyed in the middle (original magnification ×750; bar=10 μm). (c) Lased calculus (using 50 mJ, 10 pps, 1 s) "score 2", (original magnification ×100; bar=100 μm). (d) Lased calculus (using 50 mJ, 20 pps, 1 s) "score 3", (original magnification ×100; bar=100 μm).

Second experiment, cementum and dentine

3 calculus-free teeth were root planed until it was judged that the cementum layer had been removed. This was confirmed by microscopic examination using ×10 magnification. 1 surface on each of three cementum covered and 3 root-planed teeth were divided into 8 areas using a sharp scraper. Each area was subjected to the laser treatment in the same manner as described for calculus. 2 healthy cementum covered teeth were selected at random and remained untreated as controls. The exact location of treated sites in relation to the calculus shape was recorded, to enable easy localization of the treated sites using the SEM even if there were no change.

Scanning electron microscopy

Immediately after completion of the laser treatment the specimens were immersed in ice-cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 2 h. Following fixation, the specimens were dehydrated using a series of graded ethanol solutions for the following durations; 33% for 4 h; 50% for 4 h; 67% for 12 h; 95% for 48 h; and 100% for 72 h. The final dehydration was carried out using hexamethyldisilazane for 36 h.

In previous SEM studies, cementum specimens showed several cracks. Some studies suggested that these cracks were produced by the coater machine. The vacuum in the machine causes the water content of the tissue to evaporate very quickly, with a tendency for shrinkage and cracks to occur. In this study, it was decided to put the speciments through consecutive concentrations of alcohol baths over long periods of time, and the final dehydration was carried out using hexamthyldisalasane, to remove the water content completely before coating. Moreover, to differentiate the laser induced cracks (if any) and vacuum induced cracks the following preliminary experiment was performed. A silicon impression was obtained from one calculus specimen. After laser exposure at 100 mJ, 20 pps for 10 s, another silicon impression was taken. Both impressions were poured by epoxy resin to obtain replica models of tooth before and after

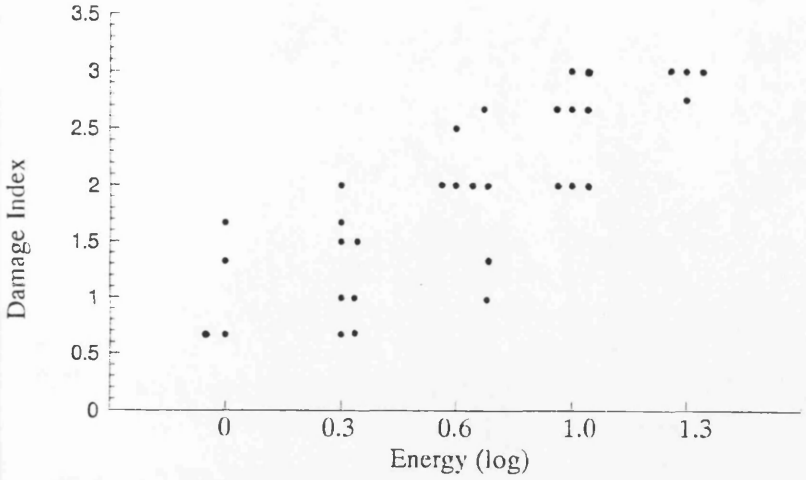

Fig. 3. Plot of log total energy versus, mean damage score.

laser treatment. Resin replicas were examined by SEM.

After cleaning with alcohol the specimens were mounted on aluminum stubs, and sputter-coated (Polaron-E5000) with gold and examined by SEM (Jeol-T300) Micrographs were taken from each treated area using ×100 and ×750 magnifications.

Index for laser damage

To quantify the effects of the laser on treated sites, the following criteria were used as an index.

Score 0. no change (Figs. 1, 2a).

Score 1. Slight changes including superficial melting. The depth of 'evaporated' (disappeared) material is less than 20 μm. These changes are not visible using ×100 magnification (Fig. 2b).

Score 2. Moderate changes, visible using ×100 magnification, shallow depression (less than 50 μm) caused by the laser (Fig. 2c).

Score 3. Severe changes, including deep craters with severe evaporation (Fig. 2d). All the specimens were examined blindly and scored using the above index.

Statistical analysis

The results from experiment no. 2 showed that there was large variability between teeth in their responses to the laser. Thus, in experiment no. 1, the tooth rather than the site was used as the unit of analysis and the index of damage was averaged over sites within a tooth to produce a single measure of damage for each tooth.

The effect on tooth damage of power setting (50 or 100 mJ), the number of pulses per second (10 or 20), and the time (1 or 5 s) was assessed by three-way analysis of variance.

The total energy input for each setting was evaluated by multiplying the power, the number of pulses and time. The relationship between the logarithm of the total energy input and damage was assessed by linear regression.

Results

The electron microscopic features of laser treated tissue seemed very typical. These features include melting down of the tooth material and calculus. The mineral seemed to melt and then resolidify. This resulted in a very porous surface. The size of pores ranged from 1 to 20 μm. In those specimens where severe damage was evident, a hole had been created which was lined with melted material.

Increasing the energy setting and duration of the laser, generally resulted in increase in the severity of damage to calculus, cementum and dentine, although it was observed that cementum and dentine were not affected using low energy settings and short durations. On the other hand, calculus was more or less affected using all energy settings and durations.

Calculus

Table 1 shows the degree of damage for each of the 94 treated sites on 32 teeth, along with the mean damage score for each tooth.

Fig. 3 shows the relationship between the log of the total energy input and the mean damage score. The least squares linear regression line was:

mean damage score $=0.925+1.54 \log_{10}$ (total energy input)

The slope of the line was significantly different from 0 ($p<0.001$) and $r^2=66\%$. A plot of residuals against the fitted values from this regression, indicated that a straight line gave an adequate fit to the data. Thus there is con-

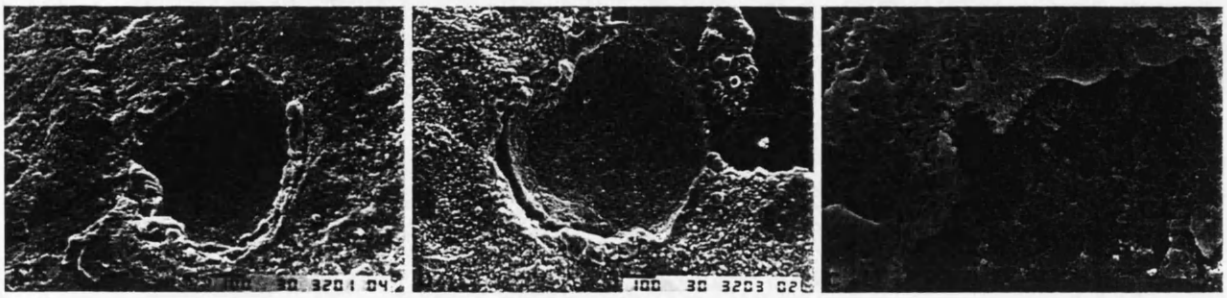

Fig. 4. Lased calculus. (a) 50 mJ, 10 pps, 1 s. (b) and (c) 100 mJ, 20 pps, 1 s. The laser has evaporated not only the calculus (CA) but also underlying cementum (CE) layer (original magnifications (a) and (b) ×100, bar=100 μm, (c) ×750, bar=750 μm).

Table 1. Distribution of degree of physical changes on calculus for each site and the mean damage score for each tooth

Laser setting	50 mJ 10 pps 1 s (group 1)					50 mJ 10 pps 5 s (group 2)					100 mJ 10 pps 1 s (group 3)					100 mJ 10 pps 5 s (group 4)				
Specimen no.	1	9	17	25	*T*	2	10	18	26	*T*	3	11	19	27	*T*	4	12	20	28	*T*
Degree of physical change																				
0			**	*	3			*	*	2		*			1					0
1	*	**		**	5		*	*		2	**	*	*	***	7					0
2	**	*	*		4	*	*	*	**	5		*	*		2		*	*	***	5
3					0	**	*			3	*				1	***	**	**		7
No. treatments	3	3	3	3		3	3	3	3		3	3	2	3		3	3	3	3	
Mean of damage	1.67	1.33	0.67	0.67		2.67	2.0	1.0	1.33		1.67	1.0	1.5	1.0		3.0	2.67	2.67	2.0	

Laser setting	50 mJ 20 pps 1 s (group 5)					50 mJ 20 pps 5 s (group 6)					100 mJ 20 pps 1 s (group 7)					100 mJ 20 pps 5 s (group 8)				
Specimen no.	5	13	21	29	*T*	6	14	22	30	*T*	7	15	23	31	*T*	8	16	24	32	*T*
Degree of physical change																				
0	*		**		3				*	1	*				1					0
1	**	*			3					0		-			0					0
2		*	*	***	5	*	***			4	*	***	***	*	8		*			1
3					0	**	*	***	*	7	**			*	3	***	***	***	**	11
No. treatments	3	2	3	3		3	3	3	3		4	3	3	2		3	4	3	2	
Mean of damage	0.67	1.5	0.67	2.0		2.67	2.0	3.0	2.0		2.0	2.0	2.0	2.5		3.0	3.0	2.75	3.0	

Table 2. Three-way analysis of variance of the effect of power setting, number of pulses per second and length of time on the mean damage score

Source	d.f.	Sum of squares	*F*	*p*
power	1	3.07	13.3	<0.001
pulses/s	1	1.96	8.4	0.008
time	1	7.91	34.2	<0.001
power×pulses	1	0.08	0.3	0.57
power×time	1	0.03	0.1	0.74
pulses×time	1	0.00	0.0	0.93
power×pulses×time	1	0.52	2.3	0.15
error	24	5.56		
total	31			

vincing evidence that higher total energy input leads to a greater mean damage score.

The effect of the power setting of the laser, the number of pulses per second and the length of time on the mean damage score were further investigated by a three-way analysis of variance. The results are shown in Table 2. None of the interaction terms are significant. However the main effects of power setting, number of pulses per second and length of time are contributing independently to the mean damage score in an additive way.

A consistent observation was that specimens with complete evaporation of calculus also showed some degree of damage to the underlying cementum (Fig. 4), however, because of the variability in calculus thickness which could not be controlled, this damage to the underlying cementum was not scored in the present study.

Cementum and dentine

Figs. 5, 6 show the degree of damage to the cementum and dentine specimens. Large variability was observed between different teeth, for both cementum and dentine specimens. For instance, tooth D was affected by none of the laser exposures, whereas tooth F was affected by all of the power settings employed.

Replica models

Replicas reproduced the surface details accurately. SEM showed no difference in cracking of the two replicas (Figs. 7, 8). Therefore, the cracking could not be attributed to the laser treatment.

Discussion

It seems that regardless of the energy and duration of the laser irradiation, there were more severe physical changes to calculus compared to either dentine or cementum. This may be due to calculus absorbing the NdYAG laser wavelength (1064 nm) more readily than the dental tissues. Although the calculus is darker in colour than dentine or cementum, greater absorption at this infra-red wavelength cannot be assumed. Even with very low energy and duration (50 mJ, 10 pps, 1 s), the laser could cause some physical changes to calculus (Fig. 2b), whereas it did not affect any of the cementum specimens using 50 mJ, 10 pps for either 1 or 5 s. On the other hand, using 100 mJ, 20 pps for 5 s the laser caused severe destruction of calculus, dentine and cementum.

Interestingly, when the laser energy was strong enough and calculus was thin enough to be evaporated completely, some parts of the underlying dental tissues were affected too. It seems that none of the laser settings could differentiate calculus and dental tissues and all were affected (e.g., Figs. 4a-c). Perhaps calculus causes more laser power absorption to the area, thus more heat is generated. This heat may affect not only the calculus itself but also the adjacent tissues. Figs. 4a, b

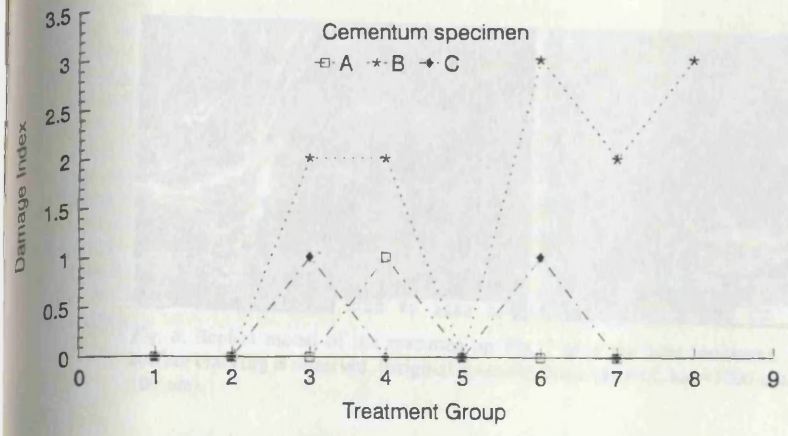

Fig. 5. The degree of damage to the cementum specimens at different laser parameters.

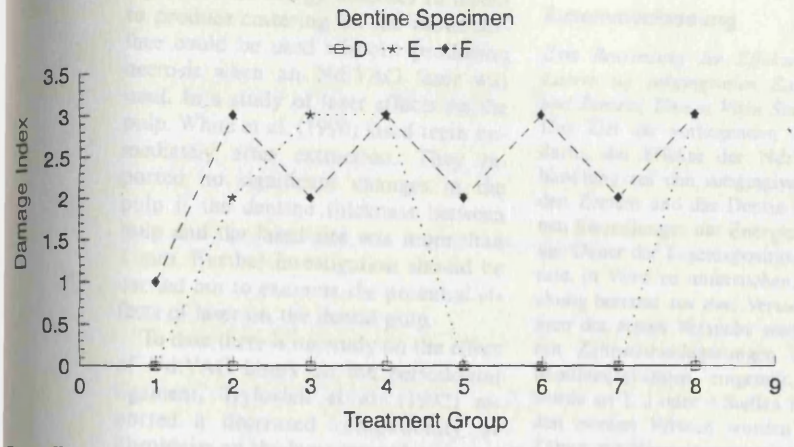

Fig. 6. The degree of damage to the dentine specimens at different laser parameters.

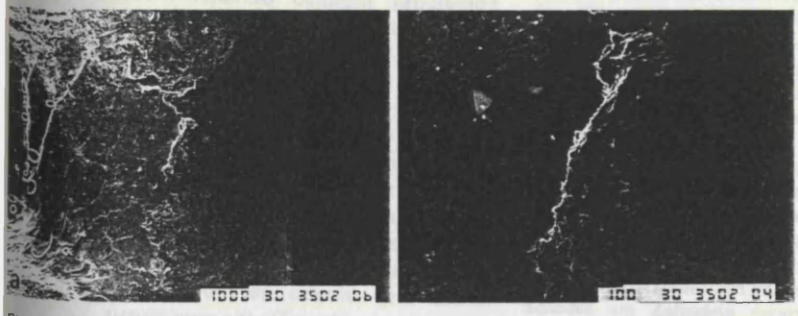

Fig. 7. Replica model of calculus before the laser treatment. A few cracks are visible on the tooth surface (original magnifications: (a) ×15, bar=1000 μm; (b) ×150, bar=100 μm).

show that there were no distinct boundaries between cementum and calculus on the internal wall of the laser lesions. No specimen was found in which the laser could evaporate calculus completely without evaporating cementum. This inability to differentiate foreign and host material may limit the clinical application of the laser for calculus removal in periodontal pockets.

Results showed that the value of power setting, number of pulses per second and radiation duration have an additive contribution to the damage score. However, the study has low power to distinguish between different models of the effect of power setting, number of pulses per second and length of time on the mean damage score in view of the small overall sample size and the arbitrary nature of the mean damage score.

This study has attempted to quantify the effects caused by the laser on the root surface. In previous studies, the laser handpiece was moved on the root surfaces with an almost parallel angulation to the root surface, where the whole surface was exposed using an overlapping movement of the laser tip. As stated previously, it may be possible that some points on the root surface had multiple treatments because of the overlapping movement. On the other hand, viewing the small size of optic fibre diameter in relation to the whole treatment area, there was a possibility of undertreating some parts. It was not clear, therefore, which part of the treated surface could be attributed to the particular laser settings. In addition, the degree of damage might have been influenced partly by the speed of movements, a factor which was not controlled in previous studies. To reduce the methodological variability, it was decided to lase only one point for a specific period of time without moving the fibre. The protocol appears to have been highly successful.

Results have indicated that specific dentine and cementum specimens appear to have different degrees of susceptibility to the laser. The variability of this susceptibility was evidenced by the two following extremes, one of the dentine specimens was not affected by any of the eight laser settings, whereas another was more or less affected by all 8 settings. Others fell somewhere between the two. This phenomenon may be the result of variability in colour, texture, % of water content, or some other inherent host or site factors which may influence the degree of energy absorption. Such unpredictable variation may limit the clinical application of lasers in periodontal pockets. Moreover, in most teeth, different sites of the same calculus specimen showed different susceptibility to laser exposure. Out of 32 teeth only 11 teeth showed equal damage scores for all treated sites. This is probably due to variation in the colour, thickness, composition, and other characteristics of calculus in the same tooth.

If lasers are to be used for any periodontal treatment, they should be harmless to pulp tissues. Adrian (1977)

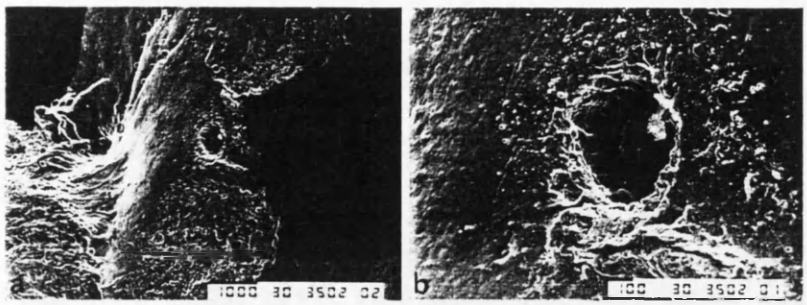

Fig. 8. Replica model of the specimen on Fig. 7 after the laser treatment. No difference in surface cracking is observed. (original magnifications: (a) ×15, bar=1000 μm; (b) ×150, bar=100 μm).

reported that energy densities sufficient to produce cratering on the tooth surface could be used without producing necrosis when an Nd:YAG laser was used. In a study of laser effects on the pulp, White et al. (1990) lased teeth immediately after extraction. They reported no significant changes in the pulp if the dentine thickness between pulp and the lased site was more than 1 mm. Further investigation should be carried out to examine the potential effects of laser on the dental pulp.

To date there is no study on the effect of Nd:YAG lasers on the periodontal ligament. Trylovich et al. (1992) reported a decreased compatibility of fibroblasts on the laser treated root surface. Moreover, in a study with cultured human skin fibroblasts, Abergel et al. (1984) reported collagen production and DNA synthesis to be delayed by Nd:YAG laser exposure. It is likely that periodontal fibroblasts show similar response after laser exposure.

Further work is necessary to clarify root tissue behaviour to the laser irradiation. In conclusion, the laser can cause physical changes on calculus under laboratory conditions, even if used with low energies and short durations. However, calculus free dental tissues were not affected by low energies of laser (50 mj-10pps-1sec). In addition, there was variability in the degree of susceptibility of different teeth and different parts of the same tooth to the laser.

Acknowledgments

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Zusammenfassung

Eine Beurteilung der Effekte eines Nd:Yag Lasers auf subgingivalen Zahnstein, Dentin und Zement. Eine in Vitro Studie

Das Ziel der vorliegenden Arbeit bestand darin, die Effekte der Nd:YAG Laserbehandlung auf den subgingivalen Zahnstein, den Zement und das Dentin bei verschiedenen Einstellungen der Energiezuführung und der Dauer der Laserexpositionen der Präparate, in Vitro zu untersuchen. Die Untersuchung bestand aus zwei Versuchen. Im Rahmen des ersten Versuchs wurden 32 Zähne mit Zahnsteinanlagerungen in 8 Laserbehandlungsgruppen eingeteilt. Jeder Zahn wurde an 2, 3 oder 4 Stellen behandelt. Für den zweiten Versuch wurden 3 extrahierte Zähne mit Wurzelzementschicht und 3, mit Wurzelglättung behandelte, extrahierte Zähne mit entblößtem Dentin ausgewählt. Eine Oberfläche an jedem Zahn wurde 8 verschiedenartigen Laserbehandlungen ausgesetzt. Bei beiden Versuchen wurden alle Präparate mit Rasterelektronenmikroskopie untersucht. Von jeder behandelten Stelle wurden Mikroradiogramme in 100- und 750facher Vergrößerung angefertigt. Um das Ausmaß des Laserschadens zu bewerten, wurde eine willkürliche Skala (von 0–3) angewandt. Im allgemeinen beschädigte der Laser den Zahnstein mehr als den Zement oder das Dentin. Die lineare Regressionsanalyse ergab, daß höhere Energiezufuhr den größeren mittleren Schaden am Zahnstein verursachte (R^2= 66%, p<0.001). Was den Zahnstein anbelangt, zeigte die 3-Wege Varianzanalyse, daß die Einstellung der Energiezuführung, die Zahl der Impulse pro Sekunde und die Dauer der Exposition, unabhängig von einander und additiv, zu der Höhe des mittleren Schadens-Score beitrugen. Die Zementpräparate wurden durch die Behandlung 1 (50 mJ, 10 pps, 1 Sek), die Behandlung 2 (50 mJ, 10 pps, 5 Sek) und die Behandlung 5 (50 mJ, 20 pps, 1 Sek), nicht beeinflußt. Die Dentinpräparate wurden durch die Behandlung 1 (50 mJ, 10 pps, 1 Sek) nicht beschädigt. Die Resultate zeigten weiterhin, daß, sowohl beim Zahnstein, beim Zement oder am Dentin, Schwankungen der Suszeptivität der einzelnen Zähne und der verschiedenen Abschnitte eines jeden Zahns vorhanden waren.

Résumé

Une évaluation des effets d'un laser Nd:YAG sur le tartre sous-gingival, la dentine et le cément: une étude in vitro

Le but de cette étude a été d'évaluer les effets du traitement au laser Nd:YAG sur le tartre sous-gingival, le cément, la dentine, *in vitro* et à divers niveaux de puissance et de durée. Cette étude comprenait deux expériences. Dans la première, 32 dents avulsées avec tartre ont été réparties en 8 groupes de traitement au laser. Chaque dent a été traitée au niveau de 2, 3 ou 4 sites. Dans la seconde expérience, 3 dents avulsées recouvertes de cément et 3 dents avulsées avec racines lissées et dentine exposée ont été sélectionnées. Une surface de chaque dent a fait l'objet de huit traitements différents au laser. Dans les 2 expériences, tous les échantillons ont été analysés au microscope électronique à balayage. Des micrographies ont été réalisées de chaque site traité, à une amplification de 100× et 750×. Une échelle arbitraire (de 0 à 3) a été utilisée pour estimer le niveau des lésions causées par le laser. Généralement, le laser entraînait plus de dégâts au niveau du tartre qu'au niveau du cément ou de la dentine. L'analyse de régression linéaire a démontré qu'un flux énergétique élevé provoque au niveau du tartre un degré moyen de lésion (R^2=66%, p<0.001). L'analyse de variance à trois voies a montré que pour le tartre la puissance, la fréquence et la durée de l'exposition ont contribué indépendamment au degré moyen de lésion, et ce, de manière additive. Des échantillons de cément n'ont pas été affectés par le traitement 1 (50 mJ, 10 pps, 1 s), le traitement 2 (50 mJ, 10 pps, 5 s) et le traitement 5 (50 mJ, 20 pps, 1s). Les échantillons de dentine n'ont pas été endommagés par le traitement 1 (50 mJ, 10 pps, 1 s). Les résultats démontrent également une variabilité de sensibilité au niveau des différentes dents et des différents sites pour chaque dent, ceci étant aussi bien valable pour le tartre et le cément que pour la dentine.

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An evaluation of the Nd:YAG laser in periodontal pocket therapy

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The aim of this study was to determine whether the Nd:YAG laser energies of 50 and 80 MJ at 10 pulses per second (pps) were capable of improving the clinical parameters associated with periodontal disease. These energy settings were chosen as previous work indicated that higher values would damage root surfaces and that 80 MJ had an in-vitro bactericidal effect. Eighty periodontally affected sites in teeth scheduled for extraction from 11 patients with adult periodontitis were randomly placed in one of the following four treatment groups: 1. laser treatment at 50 MJ, 10 pps for 3 minutes; 2. laser treatment at 80 MJ, 10 pps for 3 minutes; 3. scaling and 4. untreated control. Probing depth, bleeding on probing (BOP), plaque index, gingival index and gingival crevicular fluid (GCF) volume were measured at baseline and week 6. Baseline subgingival microbiological samples were collected, then repeated immediately after treatment and at week 6 to assess the total anaerobic colony forming units (CFU). Only the scaling group showed a significant reduction in pocket depth and BOP ($P < 0.001$). The microbial samples taken immediately after scaling and laser at 80 MJ and 10 pps treatments showed a significant reduction in total CFU compared to the baseline ($P < 0.01$), which was sustained only in the scaling group until week 6. Electron microscopy did not reveal any heat damage on the root surfaces. This study demonstrated that application of Nd:YAG laser pulses of 50 MJ and 80 MJ failed to improve the clinical and microbiological parameters of periodontal disease.

Recently, extensive claims have been made for the pulsed Nd:YAG laser which include efficacy in calculus removal, pocket curettage, pocket depth reduction and treatment of dentinal hypersensitivity.[1–4] In addition, some investigators have suggested that lasers can reduce the bacterial population in the periodontal pocket.[5]

The main strategy in periodontal therapy is the elimination of bacterial plaque from periodontal pockets and root surfaces. Laser radiation of various wavelengths including Nd:YAG laser has been shown to be capable of killing bacteria[5–8] and

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so in theory, could be used to render the root surface free of bacterial plaque and deposits. The Nd:YAG laser, however, has been shown to alter the physical and biological properties of the cementum surface, if used at energies in the range recommended by the manufacturer.[5,9–12] Our group has examined the in-vitro effect of this laser on calculus (fixed fibre, few seconds exposure) and found that there was a wide variability among the specimens in terms of the degree of physical changes on calculus following laser application.[13]

Root surface damage has been reported to be caused by energy level of 80 MJ at 10 pps *in vitro* using a moving fibre.[10] Cobb *et al.*[5] used energy settings of 1.75 W (about 87 MJ, 20 pps) and higher in vivo, and reported a significant reduction in the proportion of periodontopathic bacteria. However, the cementum surface was damaged by the high levels of laser energy as demonstrated by scanning electron microscopy (SEM). It has also been reported that the Nd:YAG laser is capable of 90% kills or more for some oral bacteria including several periodontopathic species at an energy setting of 80 MJ and 10 pulses per second (pps) for 3 minutes in vitro where the fibre was manipulated within 50 μl microbial suspensions containing 10^8 CFU/ml.[14]

The aims of this study were: 1. to determine whether the Nd:YAG laser energy settings of 50 and 80 MJ at 10 pps could change the clinical parameters associated with chronic periodontitis; 2. to check if these laser energy levels damaged the cementum surface *in vivo*; and 3. to determine the effect of the laser on the total viable anaerobic counts in the periodontal pocket.

Materials and methods

Subjects

Eleven patients with untreated chronic adult periodontitis who had periodontally affected teeth of poor prognosis scheduled for extraction took part in this study. In total 80 sites were selected, each receiving one of the four treatments described subsequently. In each patient four pockets were matched for pocket depth within a tolerance of 1 mm. Nine patients provided eight sites, that is two sites for each treatment modality, and two patients provided one site for each treatment.

Study design

The study design is shown in figure 1. The following clinical parameters were recorded for each site before treatment and 6 weeks after treatment: plaque index,[15] modified gingival index,[16] gingival crevicular fluid volume determination using filter paper strips and Periotron 6000,[17] bacterial sampling, pocket

depth using a pressure sensitive probe with a force of 25 g and the presence or absence of bleeding on probing.

Treatments

Treatments included laser treatments of 50 MJ, or 80 MJ, or scaling; one site was the untreated control. The Nd:YAG laser used has a wavelength of 1064 nm. In this study the laser energy was delivered in a pulsed mode with a pulse duration of 150 μs via an optical fibre of 320 μm diameter. Laser treatments were performed with a Nd:YAG laser (American dental laser, model d.lase-300, Sunrise Technology Inc., Fremont, California) with a contact handpiece, at power settings of either 50 MJ or 80 MJ both at 10 pulses per second (pps). The energy density was 62.9 J/cm^2 and 99.5 J/cm^2 for the two laser settings used in the study respectively. The optical fibre was kept almost parallel to the root surface with the fibre tip in contact with the root surface during treatment. This means that a diverging beam was incident on the root surface. The handpiece was moved back and forth by the operator to cover the whole surface area of each pocket for 3 minutes. The total energy dose delivered to the root surface were 90 J and 144 J for the two laser settings respectively. Scaling and root planing were performed with periodontal hoes and curettes until a smooth surface was obtained (average of ten overlapping strokes). Laser treatments and scaling were preceded by local anaesthetic administration. Control treatment included applying the laser handpiece to the root surface of control sites for 3 minutes with the laser machine switched off, taking into account any effect of the fibre mechanically removing bacteria.

Bacteriology

Microbial samples were taken from each site before, immediately following and 6 weeks after treatment. Samples were collected by first removing supragingival plaque, then isolating the site with cotton rolls. A sterile, medium-size paper point was inserted to the depth of the pocket for 10 seconds and transferred into a bottle containing Fastidious Anaerobe Broth (FAB) (Lab M, England) and immediately transferred to the laboratory for total anaerobic count (CFU/ml). Each sample was Vortex mixed for 30 seconds, serially diluted in FAB from neat to 10^{-5} and inoculated with a spiral plater (Don Whitley Scientific, Shipley, England) onto Fastidious Anaerobe Blood agar containing 7.5% v/v sterile defibrinated horse blood. Plates were incubated for 10 days at 37°C in an anaerobic incubator (Don Whitley Scientific, Shipley, England) at an atmosphere of 5% H_2, 10% CO_2, 85% Nitrogen. The total number of colony

forming units in each sample (CFU/ml) was calculated and converted to logs for statistical analysis.

Scanning electron microscopy (SEM)

After the 6-week visit, laser treated teeth were extracted to complete the patient's treatment plan. The extracted teeth were then fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4. Following fixation, the specimens were dehydrated using a ascending series of graded ethanol solutions and the final dehydration was carried out using hexamethyldisilazane for 36 hours. The specimens were sputter-coated (Polaron E5000) with gold and viewed on a Jeol JSM T300 SEM at 30 kV to examine whether the laser damage was present on the root surfaces. The characteristic features of the laser damaged surface include charring, crater formation, cementum meltdown with subsequent resolidification of inorganic material.[5,10,13]

Statistical analysis

For the analysis of plaque and gingival index data, baseline and 6-week visit data were compared using Wilcoxon's test and changes in these parameters in the four groups were subjected to Friedman's test. The latter compares the mean ranks of treatments ie an average of every site's score, ranked across the four treatment groups within each patient. BOP data of baseline and 6-week visit in each treatment group were compared using the McNemar test. Pocket depths were compared using a paired t-test on baseline and 6-week visit data for each treatment group. Repeated measurements analysis of variance (ANOVA) was used to compare pocket depth changes in four groups, and where an overall significant difference was detected, Duncan's multiple range test was used to uncover the significantly different groups. For the microbiology data, differences between the counts in samples before and immediately after treatment, as well as differences between the counts before treatment and at the 6-week visit, were subjected to repeated measures ANOVA and Duncan's multiple range test.

Results

Overall the plaque index scores recorded for all sites were relatively low during the study (0.6 at baseline and 0.37 at 6-weeks). There was a slight reduction in plaque scores at the 6-week visit compared to baseline for all four groups, although only the control group showed a significant reduction (P = 0.043, Wilcoxon's test). Friedman's test on changes in plaque scores among the four groups showed no significant differences (P = 0.94) (Table I). The gingival index also was

slightly reduced in all groups over time with the highest reduction being recorded in the scaling group (mean reduction 0.5) followed by the laser (50 MJ), laser (80 MJ) and control groups with mean reductions of 0.26, 0.05 and 0.05 respectively. No significant difference was found in changes of gingival scores among the four groups ($P = 0.29$) (Table II).

All groups showed a reduction in pocket depth at the 6-week visit compared to baseline (fig. 2), but only the scaling group showed a significant reduction ($P < 0.001$, paired t-test). Repeated measures analysis of variance on differences of the four groups proved to be significant ($P = 0.003$). In addition, Duncan's multiple range test revealed that scaling was significantly superior to all other groups ($P < 0.05$).

The total anaerobic counts of subgingival plaque samples collected immediately after scaling and laser (80 MJ) treatments showed a significant reduction compared to those collected before treatment ($P < 0.01$, paired t-test; fig. 3). However, after 6 weeks only the scaling group still showed a significant reduction compared to baseline ($P < 0.01$, paired t-test). The differences in counts between the baseline and post-treatment samples and between baseline and 6-week samples were subjected to repeated measures ANOVA and a significant difference was found for the former comparison ($P < 0.001$). Duncan's multiple range test elucidated a significant difference between scaling and all other groups ($P < 0.05$). However, differences between baseline and 6-week visit samples among the four groups were not significantly different ($P = 0.059$).

No improvement was observed in bleeding on probing (BOP) for any of the laser treated sites throughout the study (fig. 4), although the control group showed a little reduction (10%). The scaling group, however experienced a 45% reduction (from 95% to 50%) which proved to be significant ($P = 0.0039$, McNemar test). In both of the laser treated groups, the gingival crevicular fluid (GCF) volume was slightly increased at 6-week compared to baseline, whereas the scaling group showed a reduction and the control group remained almost unchanged (fig. 5). None of these changes, however, reached statistical significance ($P < 0.05$).

Scanning electron microscopy

SEM examination of laser treated teeth showed no evidence of damage attributable to the laser irradiation.

Sensitivity

Five patients presented with mild hypersensitivity to hot and cold drinks on treated teeth one week after treatment. Of these, three pa-

tients had hypersensitivity related to scaling, laser (50 MJ) and laser (80 MJ). One patient had sensitivity on scaling teeth and laser (80 MJ) treated teeth, and in another patient the laser (50 MJ) and laser (80 MJ) treated teeth were sensitive.

Discussion

Most treatment modalities used in periodontal therapy aim to reduce the bacterial plaque on the root surface and periodontal tissues to levels compatible with the host response such that the disease will be controlled. The effectiveness of scaling and root planing in the treatment of periodontal disease is universally accepted.[18,19] Adjunctive therapy aimed at reducing or eliminating bacteria such as locally delivered antibacterial agents has proved useful in periodontal treatment.[20–22] In theory, the ability of laser energy to kill and ablate bacteria might be useful in eliminating bacterial plaque and thereby reducing probing depth and bleeding on probing. The purpose of the present study was to determine whether the relatively low level laser pulse energies were sufficient to reduce microbial numbers, and improve the clinical parameters of periodontal disease and to determine whether these energy levels had any detrimental effect on the root surface *in vivo*.

All groups (including the untreated control) showed some reduction in pocket depth at the re-assessment visit compared to baseline. The slight reduction in pocket depth in all groups may be explained by the improved oral hygiene of the patients. Three weeks before baseline, they received a full oral hygiene instruction. Furthermore, after baseline, there was still a slight improvement in the plaque scores for all groups, however, supragingival plaque control has been shown to have a minor effect on pocket depth.[23]

The patients had a relatively low gingival index at the baseline (mean = 1.77) and the scaling group showed the highest reduction in gingival index (0.5) and bleeding on probing, whereas the reduction in the gingival index of the other groups was minimal and could be attributed to improved oral hygiene. While the gingival index may reflect the presence of inflammation at the periphery of the periodontium, the absence of bleeding on probing may provide a better assessment of health for the whole periodontium. Only the scaling group showed a significant reduction in the incidence of bleeding on probing, suggesting that the laser treatments used in this study were not capable of reducing the level of inflammation within the pockets.

The post-therapy reduction in anaerobic counts in the scaling group was significantly greater than all other groups; laser (80 MJ) treatment produced only a three-fold reduction in bacterial numbers which failed to change significantly the clinical parameters during the course of the study.

In this study, lower pulse energies than those used by Cobb *et al.*[5] were used. This was done to limit any thermal effect which laser treatment may have on the cementum and to test whether 80 MJ pulses (3 min exposure) which yielded 99.9% kills for some species[14] were as successful in inhibiting bacteria in vivo. In previous studies, different methods and microbial volumes have been investigated,[5,14] making determination of the energy dose per microbial cell difficult. This also makes determination of the optimal laser parameter for bacterial killing difficult.

SEM examination of the laser treated surfaces in this investigation revealed no sign of physical damage due to laser energy. This observation is contrary to that reported by Trylovich *et al.*[10] who used an energy setting of 80 MJ and 10 pps for 1 minute in vitro and observed by SEM physical damage on the root surface. This difference may be due to lasing technique since the handpiece was held perpendicular to the root surface by Trylovich *et al.*[10] but almost parallel to the surface in the present study. The laser energy delivered to the root surface is higher if the fibre is held perpendicular to the root surface. Moreover, the presence of biological fluid in the pocket could have a cooling effect on the root surface and might prevent root damage. The root surface adjacent to the pocket is located subgingivally and in order for the laser energy to have access to the surface, the handpiece has to be kept almost parallel to the surface.

In the present study, teeth with a poor prognosis were used because there was no certainty as to whether the laser energy would have a permanent detrimental effect on the root surface, pulp or periodontium. The observation of tooth hypersensitivity to cold and heat in some of the laser treated teeth may be due to either damage to the cementum and exposure of dentinal tubules or damage to the pulpal tissues because of laser radiation penetrating through the cementum and dentine. SEM examination did not show cementum surface damage, and this may raise concern about the pulpal effect of the laser energy. The Nd:YAG laser energy has a wavelength of 1064 nm and is capable of penetrating through tissues to a considerable depth. Clearly, further research is required to determine a clinically effective and yet safe method for applying laser energy in the treatment of periodontally diseased tissues.

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Table I Plaque index at baseline and 6-week visits among the different treatment groups (mean ± standard error of the mean are displayed)

Treatment	Baseline	6 week	Change*
Scaling and root planing	0.65 ± 0.18	0.50 ± 0.17	0.15 ± 0.21
Laser (50 MJ)	0.80 ± 0.21	0.45 ± 0.14	0.35 ± 0.23
Laser (80 MJ)	0.50 ± 0.14	0.40 ± 0.15	0.10 ± 0.12
Untreated control	0.45 ± 0.15	0.15** ± 0.11	0.30 ± 0.13

*$P = 0.939$, Friedman's test.
**Significantly different from baseline; $P = 0.043$, Wilcoxon's test.

Table II Modified gingival index at baseline and six-week visits among the different treatment groups (mean ± standard error of the mean are displayed)

Treatment	Baseline	6 week	Change*
Scaling and root planing	1.65 ± 0.18	1.15 ± 0.15	0.50 ± 0.24
Laser (50 MJ)	2.05 ± 0.15	1.80 ± 0.17	0.25 ± 0.14
Laser (80 MJ)	1.95 ± 0.15	1.90 ± 0.14	0.05 ± 0.15
Untreated control	1.45 ± 0.15	1.40 ± 0.18	0.05 ± 0.22

*$P = 0.299$, Friedman's test.

Fig. 1 Study design.

Fig. 2 Pocket depth at the baseline and 6-week visits among the treatment groups (mean and 95% confidence intervals are displayed).

Fig. 3 Total anaerobic count (log) at baseline, immediately after treatment and 6-week visit among the treatment groups (mean and 95% confidence intervals are displayed).

Fig. 4 Percentage of sites with bleeding on probing at baseline and 6-week visit among the treatment groups.

Fig. 5 Change in the gingival crevicular fluid volume after treatment among the treatment groups (mean and 95% confidence intervals are displayed).

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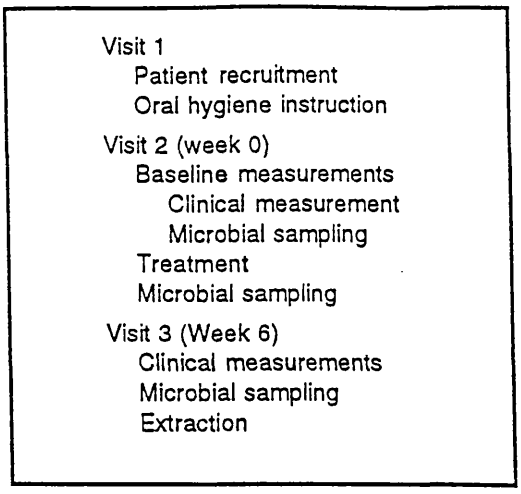
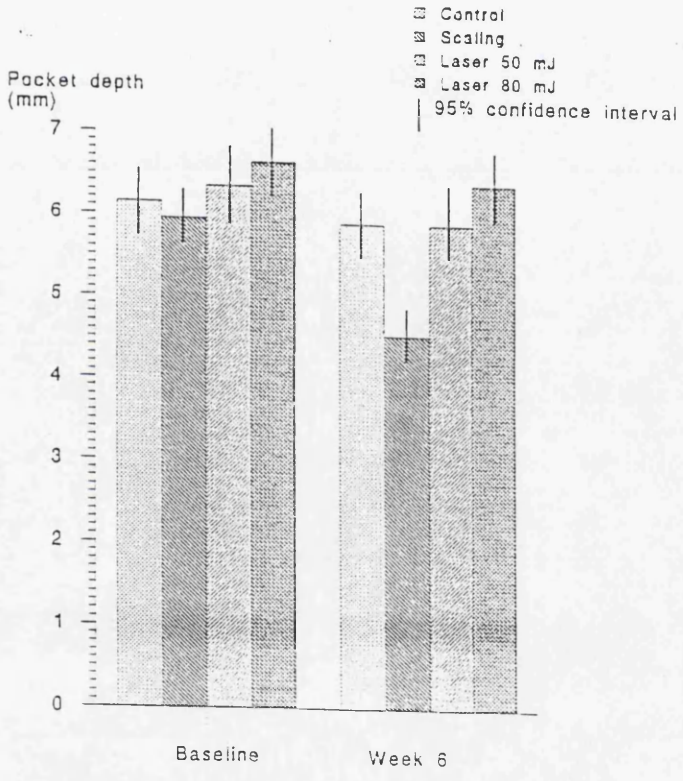
Figs 1-5




Control
Scaling
Laser 50 mJ
Laser 80 mJ
95% confidence interval
Pocket depth
(mm)
7
6
5
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2
1
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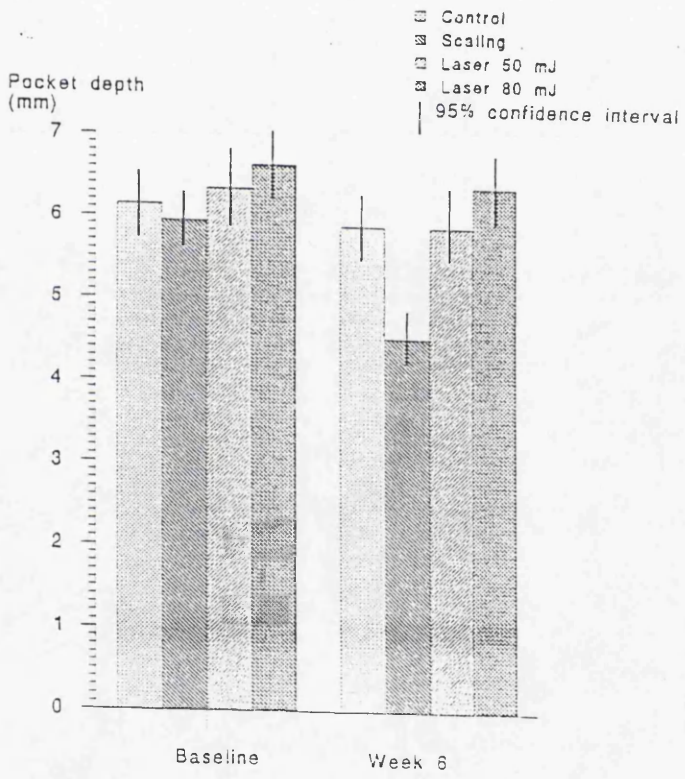
Baseline
Week 6


Control
Scaling
Laser 50 mJ
Laser 80 mJ
95% confidence interval
Pocket depth (mm)
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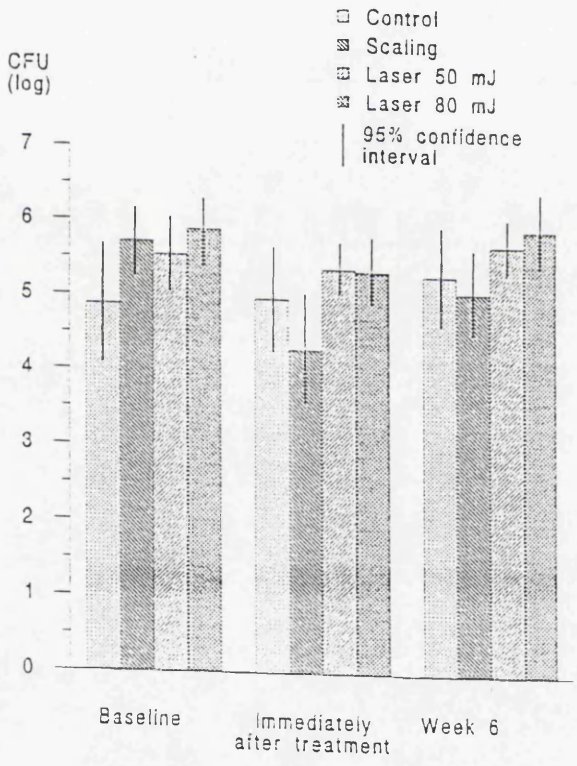
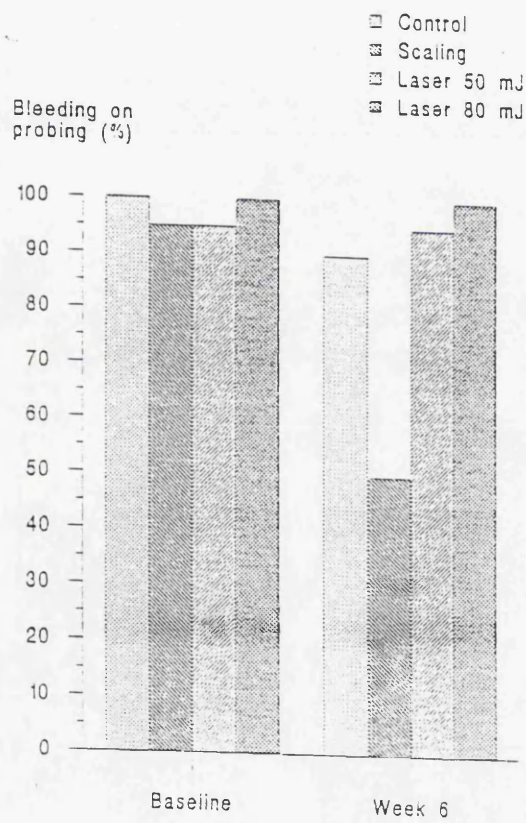
Baseline
Week 6


Control
Scaling
Laser 50 mJ
Laser 80 mJ
95% confidence interval
CFU (log)
7
6
5
4
3
2
1
0
Baseline
Immediately after treatment
Week 6


Control
Scaling
Laser 50 mJ
Laser 80 mJ
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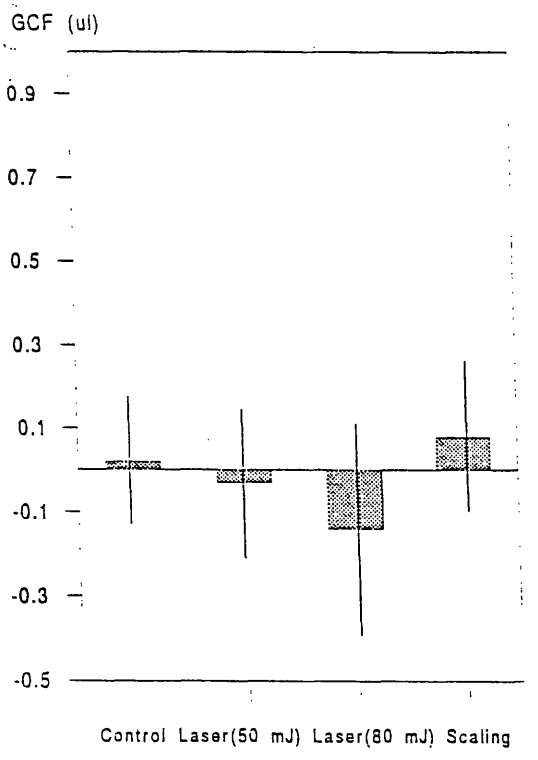
90
80
70
60
50
40
30
20
10
0
Baseline
Week 6


GCF (ul)
0.9
0.7
0.5
0.3
0.1
-0.1
-0.3
-0.5
Control
Laser(50 mJ)
Laser(80 mJ)
Scaling

A Comparative Evaluation of 3 Locally Delivered Antimicrobial Systems Used in Conjunction with Scaling and Root Planing in Persistent Periodontal Pockets

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Keywords: Locally delivered, Metronidazole, Tetracycline, Minocycline, Periodontitis.

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Abstract

The aim of this study was to evaluate the efficacy of 3 commercially available locally delivered antimicrobial systems as adjuncts to scaling and root planing in treatment of sites with persistent periodontal lesions following a course of scaling and root planing. Fifty-four patients with 4 pockets ≥ 5 mm and bleeding on probing and/or suppuration were randomised in 4 treatment groups including: scaling and root planing plus application of 25% tetracycline fibre (S+Tet) (13 patients), scaling and root planing plus application of 2% minocycline gel (S+Min) (14 patients), scaling and root planing plus application of 25% metronidazole gel (S+Met) (14 patients) and one group received scaling and root planing alone (S) (13 patients). Clinical measurements were taken at baseline and 6 weeks after treatments. All treatments were applied using the distributors recommended protocols and resulted in significant improvement in pocket depth, attachment level, bleeding on probing and the Modified Gingival Index (MGI) scores. The improvements in clinical parameters were greater in all 3 adjunctive treatment groups than scaling and root planing alone. The mean pocket depth reductions were: S+Tet=1.35mm, S+Met=0.95mm, S+Min=0.87mm and S=0.60mm. The pocket depth reduction was significantly greater in scaling plus tetracycline fibre group than scaling and root planing alone group (p=0.002). The difference between groups in improvement of attachment level or bleeding on probing was not significant. Scaling plus tetracycline fibre treatment resulted in the greatest reduction in the MGI scores which was significantly greater than all other groups. While the frequency of sites with suppuration was markedly reduced following all treatments, it reached zero in the scaling plus tetracycline fibre group. No serious adverse effect was observed or reported in any treatment group. While all 3 locally applied antimicrobial systems seem to offer some benefit over scaling and root planing alone, a treatment regime of scaling and root planing plus tetracycline fibre placement gave the greatest advantage in the treatment of persistent periodontal lesions at least during the 6 week period following treatment.

Introduction

Currently, the primary objective of periodontal treatment is to stop disease progression. The results of several clinical trials indicate that meticulous scaling and root planing in conjunction with patient's proper plaque control, can arrest periodontitis.[1-5] Nevertheless, scaling and root planing may not always result in a complete elimination of the disease. Poor access to the bottom of deep pockets, and anatomical complexity may occasionally limit the efficacy of root planing.[6,7] It has also been suggested that repopulation of scaled teeth from the bacterial reservoirs in dentinal tubules may be responsible for recurrence of the disease.[8] Moreover, some suspected periodontal pathogens have been shown to invade the soft tissue adjacent to pockets,[9] an area which may not be debrided by root planing.

The recognition of bacterial plaque as the main etiologic factor in the pathogenesis of periodontal disease, encouraged many investigators to use antibiotics as adjuncts to mechanical treatment of periodontitis (reviewed by Slots and Rams).[10] The results of clinical trials on the use of antibiotics as the sole treatment or adjunct to mechanical therapy for untreated periodontitis are controversial.[11-14] Moreover, the well known unwanted effects associated with the systemic use of antibiotics preclude the use of these agents as the sole or adjunctive treatment for untreated periodontal lesions as a routine procedure.

To overcome the considerable disadvantages of systemic antibiotic therapy, local delivery of antibacterial agents into periodontal pockets has been extensively developed and investigated since the late 70's. Many systems were designed to maintain high levels of antimicrobial agents in the crevicular fluid with minimal systemic uptake.[15-20] Many clinical studies have been conducted to assess the value of these systems as adjuncts to root planing or the sole treatment for untreated periodontal lesions.[21-26] If these systems are used as adjuncts to scaling and root planing, studies suggest that there is a

slight advantage over scaling and root planing alone, although this difference has often been clinically minimal or statistically insignificant. This may be because scaling and root planing alone is usually quite effective in producing clinically obvious and statistically significant improvements. On the other hand, few studies have evaluated the effects of local drug delivery systems on sites which responded poorly or showed recurrence of disease after scaling and root planing.[27,28] However, to date, there has been no report assessing the comparative efficacy of a number of currently marketed systems using the distributors recommended protocols, on sites with previously unsuccessful mechanical therapy. We report here the baseline to six week results of an investigation into the effect of 3 commercially available local drug delivery systems on the clinical parameters of sites with persistent periodontal lesions following a course of scaling and root planing.

Materials and Methods

Study Design

This was a parallel designed study. Patients with persistent pockets which did not respond favourably to scaling and root planing, were randomised in 4 treatment groups. One group received scaling and root planing only whereas the other three groups received scaling and root planing plus one of the 3 locally delivered antimicrobial systems adjunctively as described subsequently. The patients were examined for the clinical parameters at baseline and 6 weeks after the last treatment.

Subjects and sites

The subjects comprised of 67 patients who had been attending the Periodontal Department of the Glasgow Dental Hospital for chronic periodontitis therapy. Of the 67 participants included in the study, 54 completed the course of study and 13 excluded themselves by failing to attend either treatment or examination visits. Of the 13 excluded subjects, 9 had not been allocated to any treatment yet, one was in the S group, one was

in S+Min group and 2 were in the S+Met group. The participants had previously received quadrant scaling and root planing under local anaesthetic, and in the spite of mechanical treatment and good oral hygiene, still had deep pockets with bleeding on probing. Patients inclusion criteria was that they had at least 4 non-adjacent teeth with probing pocket depth $\geq$ 5 mm with bleeding on probing or suppuration. Sites with furcation lesions were not selected. Patients with a systemic disease, or with a history of systemic antibiotic therapy over the past 6 months were excluded. The patients fulfilling the inclusion criteria were informed of the study and having given consent, alginate impressions were taken and soft occlusal acrylic stents were fabricated for the measurements of attachment levels.

Clinical Measurements

At the baseline visit and 6 weeks after the last treatment, the following clinical parameters were measured by a single examiner (MR). The Plaque Index [29], the Modified Gingival Index [30], duplicated probing pocket depth using the Florida Probe [31] (Florida Probe Corporation, Florida, USA) with a controlled force of 20 g, duplicated attachment level recordings using the Florida Probe and occlusal stents. The examiner was blind with respect to the readings which appeared on a computer monitor hidden from the examiner and recorded by an assistant. The presence of suppuration was assessed using a ball burnisher. Bleeding on probing was assessed using the Florida Probe and the presence or absence of bleeding up to 30 seconds after probing were recorded.

Treatments

The 4 study sites in each patient received one of the 4 treatments. Moreover, full-mouth scaling and root planing was performed under local anaesthesia for all patients. For each antibiotic treatment the manufacturer's/distributors recommendations were rigidly adhered to. These included: 1) Scaling and root planing alone. Each tooth with deep pocketing was root planed under

local anaesthesia for approximately 5 minutes. 2) Scaling and root planing plus application of 2% minocycline gel (Dentomycin, Cyanamid, Lederle, U.K). After scaling and root planing as described above, the minocycline gel was directly applied into the pockets using a disposable applicator until it was overfilled. The gel application took a few seconds per tooth. The gel application was repeated 2 weeks and 4 weeks after the first application. 3) Scaling and root planing plus application of 25% tetracycline ethylene vinyl acetate fibres (Actisite, Alza Corporation, Palo Alto, CA.). After full-mouth scaling and root planing as described above, the fibres were inserted into the pockets using dental floss and a plastic instrument. On average, 11.51 cm of fibre was required to fill each pocket. This was equivalent to a dosage of 5.75 mg tetracycline per pocket. The fibre placement took on average, 8.2 minute per tooth. Then the fibres were secured using a cyanoacrylate adhesive (Octyldent, Tri-point Medical LP. Raleigh NC) and a periodontal dressing (Coe-pak, GC America INC, Chicago, IL). After 10 days the patient was recalled and the fibres were removed. 4) Scaling and root planing as described above plus application of 25% metronidazole gel (Elyzol, Dumex, Denmark). After root planing, the metronidazole gel was applied into the pockets using a disposable applicator until it was overfilled. The gel application took a few seconds per tooth. The application was repeated 7 days later. All the treatments were performed by a single operator (NP). At the first post-operative visit, the patients were examined for any adverse signs or symptoms related to these treatments.

Statistical analysis

For each visit, duplicated recordings of pocket depth and attachment level measurements were averaged for each site. For pocket depth and attachment level data, the subject-based analysis was performed, i.e., the site values within each patient were averaged for each visit producing a single figure for each patient. For each treatment group, the baseline and 6 week pocket depth and attachment level data were subjected to a paired-t test.

The changes in the pocket depth and attachment level were subjected to General Linear Model (GLM) procedures with the baseline probing depth as a continuous covariate. This took into account the effect of baseline probing pocket depth on the treatment outcome. The chi-square (χ^2) test was then used to examine the association between the treatment and the cessation of bleeding on probing after treatment. Since prerequisites for the chi-square test were not met for the suppuration data, the Fisher's exact test was used to compare 2 treatments at a time. The Plaque index and the Modified Gingival Index data were analysed by the Wilcoxon's test for each treatment. The change in the indices were compared across the treatment groups using the Kruskal-Wallis test. Where there were significant differences, post-hoc comparisons were performed using multiple Mann-Whitney-U tests and the significance level was corrected using the Bonferroni adjustment for multiple comparisons. All the statistical procedures were performed using one software package (Minitab, release 9.2).

Results

In all 4 treatment groups pocket depth decreased significantly at week 6 compared to baseline (Table 1). Mean reduction in pocket depth at week 6 was greatest in patients who received scaling plus tetracycline fibre treatment and was followed by the scaling plus metronidazole gel, scaling plus minocycline gel, and scaling alone groups respectively. A primary analysis revealed that there was no significant interaction between the covariate (baseline pocket depth) and the treatment effect. Analysis of variance (GLM) showed that there was a significant difference among the treatments (p=0.011). Pair-wise comparisons revealed that pocket depth reduction was significantly greater in the scaling plus tetracycline fibre group than scaling alone (p=0.002). The remaining comparisons were not significant.

Table 2 shows the results of attachment level changes. Since the attachment levels were measured from an arbitrary reference point on the occlusal

stents, only the actual changes would be meaningful and are presented here. All treatments resulted in a significant attachment gain compared to baseline. Primary analysis showed no indication of significant interaction between the treatment type effect and the baseline pocket depth. The difference between the treatment groups did not reach statistical significance (p=0.062).

The bleeding on probing decreased significantly in all treatment groups ($p<0.001$, Fisher's exact test for paired data). The greatest reduction occurred in the scaling plus tetracycline group. However, no significant difference existed between treatments (χ^2= 2.83, d.f.=3) (Table 3).

All treatments reduced the frequency of sites with suppuration (Table 4). With the scaling plus tetracycline fibre treatment the frequency of suppuration reached zero at the reassessment visit. However, pair-wise comparisons between treatments revealed no significant difference between groups.

The study population maintained a relatively good oral hygiene and the mean PI scores remained below 1 in all treatment groups throughout the study period. The PI scores did not differ significantly after the various treatments. There was no significant difference in the PI scores among the treatment groups at baseline (p=0.570) and after 6 weeks (p=0.259).

The Modified Gingival Index (MGI) scores decreased significantly after all treatments (Table 5). The greatest reduction occurred in the scaling plus tetracycline group. The change in the MGI scores in the scaling and tetracycline group was significantly greater than the other groups. The remaining pair-wise comparisons were not significant.

No serious adverse effect was noted throughout the study. However, in some cases gingival redness was observed upon the fibre removal (5 patients), slight pain on the first day of fibre therapy was reported (1 patient), as was

bad taste on the first day of gel therapy (1 patient in the minocycline gel and 2 patients in the metronidazole gel groups) and gingival tingling on the first day of gel therapy (1 patient in the minocycline gel group).

Discussion

This study evaluated the clinical response to 3 locally delivered antimicrobial systems as adjuncts to scaling and root planing. The participants in this study had already been treated for their chronic periodontal disease using quadrant scaling and root planing under local anaesthesia and still had pocketings $\geq$ 5 mm with bleeding on probing or suppuration.

All treatment modalities used in this study including scaling and root planing alone, resulted in a significant pocket depth reduction and clinical attachment gain. In addition, other clinical parameters including BOP, suppuration and the MGI scores were significantly improved by all the treatments. The results of our investigation support the hypothesis that many sites which do not respond favourably to initial mechanical periodontal therapy, may still benefit from further episodes of scaling and root planing. These results are consistent with previous reports by Listgarten *et al.*[32] and Magnusson *et al.* [33] Scaling plus tetracycline fibre treatment produced the greatest improvement in the study sites. Pocket depth reduction was significantly greater in the scaling plus tetracycline group compared to scaling alone. Heijl *et al.*[25] reported that scaling plus tetracycline fibre produced a greater pocket depth reduction than the scaling alone, however, the difference between groups were not significant in that study. On the other hand, Newman *et al.*[28] reported an advantage in combined scaling and tetracycline fibre therapy over scaling alone on a maintenance population, which was significant 6 months after treatment.

All adjunctive antimicrobial treatments in this study produced greater mean improvements in all clinical parameters than scaling alone. This suggests

that at sites with persistent periodontal disease despite previous mechanical therapy, adjunctive local antimicrobial treatment is effective. While this is the first report which describes the effect of adjunctive use of metronidazole gel vs. scaling and root planing alone, previous studies indicated that the metronidazole formulation alone may produce improvements comparable with those of scaling and root planing alone in probing depth and bleeding on probing.[22,34] The combined treatment of scaling and root planing and minocycline gel has previously been reported to have a significant benefit over the scaling and root planing alone in the treatment of untreated periodontal lesions.[23]

The treatments resulted in a marked reduction in the frequency of suppuration among the sites. However, only in the scaling plus tetracycline fibre group did it virtually reach zero. The sustained concentration of tetracycline at very high levels [35] for 10 days could explain the superiority of this treatment modality over other locally delivered antimicrobials in the treatment of suppuration and chronic abscesses.

This was a parallel-designed study in order to disclose true differences between treatment modalities which may be associated with a considerable carry-over effect. If a split mouth design was used, the wash-out of antimicrobial agents through saliva could affect the microbial flora in study sites which received other treatments. This, in addition to boosting the systemic response due to removal of antigenic challenge or the inoculation effect following mechanical instrumentation, could mask true differences between groups if a split mouth design was used.[36]

The treatments did not result in any serious adverse effects and were all well tolerated by the patients. The adverse effects of any therapeutic agent may be reduced if delivered directly to the site of action. Adverse effects may follow systemic antibiotic therapy such as pseudomembranous colitis or

allergic reactions and tend to question the justification for systemic antibiotic therapy for a chronic and non-lethal infection such as periodontal disease. These concerns would be markedly reduced if local antimicrobial agents could be used effectively. Nevertheless, the chance of unwanted effects such as gastro-intestinal disturbances, allergic reactions and development of antibiotic resistance could not be ruled out.

The application of tetracycline fibres required 8.2 minute per tooth on average in addition to scaling time and was markedly more time consuming than other treatments. In addition, all 3 antimicrobial treatments required 2-3 visits. These factors need to be considered in the cost-benefit analysis of antimicrobial treatments over traditional approaches.

Although a significant improvement was achieved as a result of scaling alone, this was lower than would be expected if performed on an 'untreated' periodontitis population. Usually, following the initial treatment, a marked pocket reduction and shrinkage occurs due to the elimination of subgingival plaque, calculus and other plaque retentive factors. Therefore, somewhat less improvement could be expected from the scaling and root planing alone if some of these factors have already been reduced by initial therapy. Accordingly, if a true difference exists between the efficacy of combined root planing plus antimicrobial treatment and scaling alone, the possibility of detecting this difference is greater if the study subjects are selected from a population which have little gingival inflammation, excellent oral hygiene, and still some pocketing, i.e., a population which scaling and root planing could do only little to further improve their gingival condition. In fact, viewing the time and cost which is involved in using different locally delivered antimicrobial systems, the use of these agents do not seem to be justified as a part of initial periodontal therapy. Instead, a scenario is suggested whereby following initial phase therapy, tetracycline fibre could be used for local sites

with remaining severe disease, in place of surgery, whereas the other systems might be applicable in less severe cases.

This report represents the initial 6-week results of our investigation on the efficacy of locally applied antimicrobial agents for the treatment of persistent periodontitis. While these short-term results suggest that antimicrobial agents may be beneficial in the treatment of periodontal lesions, the evidence is needed for longer term benefits over 3 and 6 months to determine the full efficacy of these treatment modalities.

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Tables

Table.1 Pocket depth analysis:

a) Baseline and 6-week pocket depth and reduction in pocket depth. Means expressed in mm and (SEM) are shown.

Treatment groups	Baseline pocket depth	6- week pocket depth	Pocket depth reduction	$p^{\dagger}$
Scaling alone (n=13)	5.47 (0.23)	4.87 (0.26)	0.60 (0.14)	0.001
Scaling & minocycline gel (n=14)	5.56 (0.19)	4.81 (0.23)	0.87 (0.13)	<0.001
Scaling & tetracycline fibre (n=13)	5.51 (0.17)	4.16 (0.13)	1.35 (0.17)	<0.001
Scaling & metronidazole gel (n=14)	5.60 (0.19)	4.65 (0.20)	0.95 (0.16)	<0.001

b) Analysis of variance (GLM) on pocket depth reductions:

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Baseline pocket depth	1	1.18	1.13	1.13	3.80	0.057
Treatment	3	3.68	3.68	1.23	4.11	0.011
Error	49	14.62	14.62	0.30		
Total	53	19.49				

c) Post-hoc comparisons:

	Scaling & tetracycline fibre	Scaling & minocycline gel	Scaling & metronidazole gel
Scaling & minocycline gel	p=0.022 (not significant)		
Scaling & metronidazole gel	p=0.045 (not significant)	p=0.726 (not significant)	
Scaling alone	p=0.002	p=0.268 (not significant)	p=0.146 (not significant)

† Paired-t test (baseline vs. 6-week)

N.B. The Bonferroni correction for multiple comparisons, produces a threshold for statistical significance of p<0.020.

Table.2 Attachment level analysis:

a) change in attachment levels among the treatment groups. Means expressed in mm and (SEM) are shown.

Treatment groups	Attachment gain	$p^{\dagger}$
Scaling alone (n=13)	0.26 (0.11)	0.032
Scaling & minocycline gel (n=14)	0.45 (0.14)	0.0056
Scaling & tetracycline fibre (n=13)	0.75 (0.12)	<0.001
Scaling & metronidazole gel (n=14)	0.57 (0.15)	0.0018

b) Analysis of variance (GLM) on attachment level changes:

Source	DF	Seq SS	Adj SS	Adj MS	F	*p*
Baseline pocket depth	1	1.52	1.44	1.44	7.21	0.010
Treatment	3	1.56	1.56	0.52	2.61	0.062
Error	49	9.80	9.80	0.20		
Total	53	12.88				

† Paired-t test (baseline vs. 6-week)

Table 3. Frequency and percentage of sites with improved bleeding on probing among the 4 treatment groups at the baseline and 6 week visits with the chi-square analysis on association of improvement in bleeding on probing with treatment.

Treatment	Sites which stopped bleeding on probing
Scaling alone (n=51)	18 (35.3%)
Minocycline gel (n=56)	22 (39.3%)
Tetracycline fibre (n=51)	26 (51.0%)
Metronidazole gel (n=55)	22 (40.0%)
Total (n=213)	88 (41.3%)
χ^2= 2.826, df = 3 (not significant)	

Table 4. Suppuration data:
a) Frequency and percentage of sites with suppuration at the baseline and 6-week visits.

Treatment	Suppuration at baseline	Suppuration at week 6
Scaling alone (n=51)	8 (15.7%)	2 (3.9%)
Scaling & minocycline gel (n=56)	4 (7.2%)	2 (3.6%)
Scaling & tetracycline fibre (n=51)	6 (11.8%)	0 (0%)
Scaling & metronidazole gel (n=55)	8 (14.5%)	1 (1.9%)

b) Matrix of pair-wise comparisons to test the association of treatment with the presence of suppuration at the 6 week visit using the Fisher's exact test (*p* values are given).

	Scaling & tetracycline fibre	Scaling & minocycline gel	Scaling & metronidazole gel
Scaling & minocycline gel	*p*= 0.272		
Scaling & metronidazole gel	*p*= 0.519	*p*= 0.382	
Scaling alone	*p*= 0.247	*p*= 0.369	*p*= 0.363

Table 5. a) The mean (SEM) baseline, 6-week scores and change in Modified Gingival Index (MGI) scores among the treatment groups.

Treatment	Baseline	6 week	Change
Scaling alone	2.25 (0.09)	1.82 (0.10)	0.43 (0.10) $p=0.001^{\dagger}$
Scaling & minocycline gel	1.98 (0.12)	1.46 (0.10)	0.52 (0.15) $p=0.003$
Scaling & tetracycline fibre	2.27 (0.09)	1.18 (0.09)	1.10 (0.13) $p<0.001$
Scaling & metronidazole gel	1.98 (0.10)	1.55 (0.10)	0.44 (0.12) $p=0.002$
p value (Kruskal-Wallis test)	0.122	0.001	0.002

b) Matrix of pair-wise comparisons using the Mann-Whitney U test:

	Scaling & tetracycline fibre	Scaling & minocycline gel	Scaling & metronidazole gel
Scaling & minocycline gel	$p=0.008^{\ddagger}$		
Scaling & metronidazole gel	$p=0.001$	$p=0.624$	
Scaling alone	$p<0.001$	$p=0.613$	$p=0.942$

† Wilcoxon's test (Baseline vs. 6-week)

‡ After the Bonferroni correction for multiple comparisons, the threshold for significance level was p=0.020.

The effect of subgingival antimicrobial therapy on the levels of stromelysin and tissue inhibitor of metalloproteinases in gingival crevicular fluid

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Abstract

Recent investigations imply that a key mechanism in the pathogenesis of periodontal disease may be the ability of oral micro-organisms to induce production and /or activation of matrix metalloproteinases (MMPs) in the host tissues. It has been suggested that the pharmacologic inhibition of MMP activity could play an important role in achieving a desirable outcome in periodontal therapy. The efficacy of locally delivered antibiotics on the level of gingival crevicular fluid (GCF) stromelysin (SL) and tissue inhibitor of metalloproteinases (TIMP) on sites with a history of a poor response to mechanical treatment was studied. 52 patients with 4 periodontal pockets $\geq$5 mm and bleeding on probing were randomised into four groups of 13 patients. One group received scaling and root planing alone and the other three groups received scaling and root planing plus a locally delivered antimicrobial system. These included 25% tetracycline fibre, 2% minocycline gel, and 25% metronidazole gel. The GCF samples taken at baseline and 6 weeks after treatments were analysed using an enzyme linked immunosorbent assay (ELISA). All treatments resulted in significant improvement in clinical parameters. The pocket depth reduction was significantly greater in the scaling plus tetracycline fibre group than the scaling alone group ($p=0.003$). GCF SL levels significantly decreased after adjunctive tetracycline fibre (paired t-test, $p=0.020$) and minocycline gel (paired t-test, $p=0.023$) treatments whereas it remained almost unchanged in the other two groups. While the GCF TIMP level did not change significantly in the scaling and root planing alone group, it significantly increased for all three adjunctive antimicrobial treatments (for tetracycline fibre $p<0.001$, minocycline gel $p=0.005$, metronidazole gel $p<0.001$). The use of adjunctive locally delivered antimicrobial systems, particularly, the tetracycline family, may offer an advantage in changing the

metalloproteinase profile of the GCF to a one more compatible with periodontal health.

INTRODUCTION

Recent investigations imply that a key mechanism in the pathogenesis of periodontal disease may be the ability of oral micro-organisms to induce production and /or activation of matrix metalloproteinases in the host tissues.[1-3] Stromelysin (SL) is a member of the matrix metalloproteinase family which may play a role in chronic inflammatory periodontitis. [4] Extracellular control of these enzymes is performed by tissue inhibitor of metalloproteinases (TIMP) [5] and during normal tissue turnover, inflammation and healing, levels of MMPs and TIMP will change. [6] The effect of treatment on the levels of MMPs and inhibitors has been to decrease collagenase activity, [7-9] to decrease the amount of collagenase and stromelysin (SL) produced, and to increase the level of TIMP.[10-11]

It has been suggested that the pharmacologic inhibition of MMP activity could play an important role in achieving a desirable outcome in periodontal therapy.[12-16] In addition to antimicrobial effects, tetracyclines have been shown to be able to inhibit metalloproteinases (MMPs). However, recent results indicate that the inhibition of MMPs by systemic tetracycline depends on the drug concentration and type[2] as well as the origin of the MMP. [13] Therefore, theoretically a great inhibitory effect on MMPs would be expected if higher concentrations of drug could be delivered to the site of action. Recently, there has been increasing interest in the use of locally delivered antibiotics in order to obtain a high concentration of the drug at the target site and to minimise potential systemic adverse effects. We therefore sought to investigate the effects of locally delivered antibiotics on the amount of fibroblast derived SL and TIMP in GCF of sites with advanced periodontal breakdown.

Materials and Methods

Selection of subjects and test sites:

52 subjects with adult periodontitis (62% Female; mean age 45 ; range 30-67) who had previously been treated with subgingival scaling and root planing, were recruited from patients in the Periodontology Unit of Glasgow Dental Hospital and School. All study subjects signed an informed consent form and a full medical history was recorded. Inclusion criteria required that subjects had no history of systemic disease which could influence the course of periodontal treatment, and that they were not on any medications such as antibiotic therapy, which could influence the manifestations of periodontal disease, within the previous 6 months.

Patients selected to take part in the study had at least four periodontal pockets equal to or deeper than 5 mm with bleeding on probing. The patients were randomly allocated into four groups of 13 patients each, three of which received scaling followed by a locally delivered antibiotic. A total of 208 sites with recurrent periodontitis were examined and treated. Two diseased sites were excluded for technical reasons, one site lost its tetracycline fibre before the 10 day placement period finished and in the scaling group a further site could not be measured. In order to increase the accuracy of the measurements taken and to simplify the harvesting of GCF, the buccal, mesial and anterior sites were preferred to the lingual, distal and posterior sites.

Study Visits

Site selection was carried out at the screening visit after a full periodontal examination using a PCP-12 probe (Ash, Densply, UK). If the patient fulfilled the criteria, an impression was taken, and an acrylic stent fabricated for attachment level measurements using the Florida probe (Florida Probe Corporation, Florida, USA).

Oral hygiene instruction was given according to individual needs, but no periodontal instrumentation was carried out at the screening visit. 10 days after the screening visit baseline measurements were taken. The plaque index (PI) of Silness & Löe (1964) [17] was recorded to assess the patient's oral hygiene, the severity of gingival inflammation was measured using the gingival index of Lobene *et al.* (1986). [18] GCF sampling, volume measurements, bleeding on probing (BOP) and suppuration were registered. Duplicate measurements of pocket depth and attachment level with an acrylic stent were carried out, using the electronic pressure sensitive Florida probe, with a constant force of 20 grams.

At the treatment visits each group received one of the following treatments:

1) Subgingival scaling and root planing under local anaesthesia and the application of tetracycline impregnated fibre (Actisite®, ALZA, corporation, Palo Alto, CA), into the pocket. The fibre was wrapped loosely around the teeth by passing it through the contact points using dental floss or a plastic instrument. The pocket was occupied by fibre without creating excessive tension in the soft tissues. Cyanoacrylate adhesive was applied to the site and a dressing (Coe-Pak, GC America INC. Chicago, IL) was placed. The fibre was removed 10 days later.

2) Subgingival scaling and root planing plus the application of minocycline dental gel (Dentomycin®, Lederle Dental Division, Gosport, Hampshire, England), to the pocket until it was overfilled. The excess was removed using cotton wool pellets. This application was repeated 14 days and 28 days after the initial application.

3) Subgingival scaling and root planing plus the application of metronidazole dental gel (Elyzol®, Dumex, Denmark), to the pocket until it was overfilled. The excess was removed using cotton wool pellets. The application was repeated 7 days later.

4) The control group received subgingival scaling and root planing alone.

42 days post treatment all the baseline measurements were repeated for each patient.

During this study other sites received conventional periodontal maintenance care.

Sampling method

The site to be sampled was isolated with cotton wool rolls and supragingival plaque was carefully removed. The region was dried with a gentle air stream and 30s later GCF was collected. A Whatman grade 4 paper strip (2 ×13 mm) was inserted into the crevice until mild resistance was felt and left *in situ* for 30s. [19] The sampling method is reliable and causes no significant disturbances of the gingival blood vessels[20]. The fluid volume on the strip was measured immediately after sampling with a Periotron 6000 (Harco Electronic, Winnipeg, Canada). The jaws of the Periotron were wiped with pure ethanol and then dried between readings. Strips were placed in individual sterile micro-centrifuge tubes and stored at -30° C until further processing was carried out. Strips were eluted into 500 μl of incubation buffer at room temperature using a rotary mixer. The strips were then discarded and the eluate aliquoted and stored at -30° C. These aliquots were subsequently analysed for the quantification of SL and TIMP.

Calibration of the Periotron 6000

In order to transform the Periotron digital readings for each paper strip into volumes, and also to verify the accuracy of the instrument, the following procedure was carried out. Known volumes of PBS plus serum (in equivalent volume) were delivered to Whatman grade 4 paper strip with a Hamilton microsyringe at a range of volumes (0.05-1 μl) then Periotron readings taken.

Each measurement was performed 3 times. 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 .

GCF Analysis

All constituents were assayed by means of sandwich enzyme linked immunosorbent assays (ELISA), based on a modification of the method of Cooksley *et al.* (1991). [21] The first antibody, Mac 78 anti-stromelysin for SL and Mac 19 anti-TIMP was coated onto a 96-well polystyrene microplate (Immulon 4, Dynatech Laboratories, Billinghurst, Sussex, U.K). The experimental sample was then added and any antigen present was captured by the immobilised antibody. The plate was then incubated with the second antibody. Biotinylated Mac 15 antibody and rabbit polyclonal antibody were used to reveal TIMP and SL, respectively followed by, in the latter case horseradish peroxide (HRP)-conjugated anti-rabbit IgG (donkey) (Jackson). The polyclonal and monoclonal antibodies were against both proenzyme, and active forms of the enzymes under investigation. The ELISA for TIMP can only detect free TIMP. [21] All the antibodies were donated by Cell Tech. Ltd. Slough, UK. Finally the plate was incubated with Extravidin-Peroxidase (Sigma). Visualisation was achieved by incubation with tetramethylbenzidine (TMB) (KPL-Dynatech) substrate, and the reaction stopped with 2.5% NaF. The plate was read at 630 nm, on a Dynatech MR 5000 plate reader. Serial dilutions of standard were run for each individual plate and their optical density (ODs) used in a linear regression analysis from which the slope and intercept were used to determine the sample concentration of SL and TIMP. Results were expressed as pg/30s sample.

Statistical Analysis

In order to identify the distribution characteristics of the data, it was inspected using histograms and it was noted that the data exhibited a markedly skewed distribution. Log transformation was successful in producing a normal distribution of data within the different groups. The differences in stromelysin levels of each subject, between before and after treatment, for each treatment group, was subjected to paired t-test. The analysis of covariance (ANCOVA) was used on changes of SL and TIMP with the baseline SL or TIMP levels as continuous covariates. If a significant difference was found between treatments, then the post-hoc comparisons were performed by comparing two groups at a time and the probability threshold for statistical significance was adjusted to 0.02 using Bonferroni correction[22]. The Mann-Whitney U test and Two sample t-test were used to analyse the baseline levels of SL and TIMP respectively between bleeding and non-bleeding sites, and between suppurating and non-suppurating sites. Comparisons of SL and TIMP levels at sites with different MGI were performed using the Kruskal-Wallis test. Data were analysed using Minitab statistical package version 9.2.

RESULTS

Clinical response following the treatments has been reported in a further manuscript (Radvar *et al.,* in press). Briefly, a significant improvement in pocket depth, attachment level, bleeding on probing (BOP) and the MGI scores occurred in all treatment groups. The tetracycline fibre treatment resulted in a significantly greater change in pocket depth than the scaling alone group ($p=0.003$) (Table 1). In addition, the scaling plus tetracycline fibre treatment resulted in a greater improvement in the MGI scores than the scaling plus minocycline gel treatment ($p=0.002$), the scaling plus metronidazole gel treatment ($p=0.007$), and the scaling alone treatment ($p=0.001$). No significant

difference existed in the MGI scores between other groups. There were no significant difference in attachment level changes and BOP changes between treatment groups. The GCF volume was reduced siginificantly only the scaling plus tetracycline fibre groups. There was no significant changes in the GCF volume after other treatments.

The mean GCF level of SL decreased in all antibiotic treated groups and this decrease was significant in the tetracycline fibre group (paired t-test, $p=0.020$) and the minocycline gel group (paired t-test, $p=0.023$) (Table 1). Paired t-test demonstrated that the level of GCF TIMP significantly increased in all antibiotic treated groups, whereas it decreased in the scaling groups, although not significantly ($p=0.49$) (Table 1). The analysis of covariance (ANCOVA) on the baseline SL was used to reduce the error resulting from the variation in the baseline. The level of SL at baseline had a significant effect on the SL change following treatment ($p<0.001$), and this effect was positive i.e. the higher the baseline SL, the higher the reduction of SL. Treatment also had a significant effect on the reduction of SL ($p=0.001$). Follow-up analysis showed significant differences between; i) tetracycline fibre and scaling alone groups ($p=0.004$); and ii) minocycline gel and scaling alone groups ($p=0.002$) (Table 2).

There was a significant difference in TIMP levels between treatment groups ($p=0.007$). Furthermore, the analysis of TIMP levels revealed that a significant difference existed between the minocycline gel and the scaling alone group ($p=0.018$), and also between the metronidazole gel and scaling alone group ($p=0.001$) (Table 3).

The level of SL significantly increased with increasing gingival inflammation ($p=0.028$). No such relationship was observed between gingival inflammation and GCF TIMP ($p=0.395$) (Table 4). The level of SL was higher in sites with bleeding on probing (Mean=20.59 pg/µl), than sites with no bleeding on

probing (Mean=18.57 pg/µl), however the difference was not statistically significant (Mann-Whitney U test, p=0.47). TIMP levels were lower in bleeding sites (Mean=291.2 pg/µl) when compared with non-bleeding (Mean=322 pg/µl) sites, however this was not statistically significant (p=0.395). The level of SL was lower in sites with suppuration (Mean=15.69 pg/µl), than sites without suppuration (Mean=21.09 pg/µl) but the difference was not significant (Mann-Whitney U test, p=0.36). The TIMP level was higher in suppurating sites (Mean=381.9 pg/µl) compared with the sites which did not show suppuration (Mean=281.1 pg/µl), but this difference was not significant (p=0.12).

DISCUSSION

This paper reports the effects of the local delivery of antibiotics, as an adjunct to periodontal treatment, on GCF levels of SL, and TIMP, in patients who had previously received periodontal treatment. The clinical results of this investigation have been reported by Radvar *et al.* (1995). [23]

Golub *et al.* (1987) [24] demonstrated that tetracyclines inhibit MMP activity by a mechanism independent of their antimicrobial activity. It was shown that the systemically administrated tetracycline could inhibit collagenases derived from inflammatory sources but inhibition of fibroblast collagenase activity needed higher concentrations than could be achieved by this method of delivery. [24-25,12-13] Therefore, it is hypothesised that locally delivered tetracyclines, due to their high local concentrations can inhibit collagenase from both origins. Metronidazole appears to reduce the prevalence of *Porphyromanas gingivalis* and spirochetes at active sites in recurrent periodontitis subjects[26]. In addition, the results of Teng *et al.* (1992) [27] suggest that metronidazole treatment can cause the reduction of both active and latent forms of gelatinase. The reduction of gelatinase activity is best explained by the antimicrobial effect of

metronidazole which appears to blunt destructive host immune and inflammatory responses.

It has been suggested that SL may act as a marker of stromal cell involvement in the process of tissue degradation. [6] Our observation that the mean level of SL decreased after antibiotic treatment could be explained by the fact that local delivery antibiotics plus scaling and root planing may change the microbial flora. This may cause a larger decrease of SL than scaling and root planing alone. However, the greatest decrease was observed in the sites treated by tetracycline or minocycline delivery systems.

Previous studies have also shown that tetracyclines, in addition to decreasing the level of periodontal bacteria, resulting in decrease of MMP activity in the gingiva, could decrease the synthesis of pro-MMPs [28-29] and also protect the endogenous MMP inhibitors (TIMP-1) and other proteinase inhibitors (α1-antitrypsin) [30] from degradation and inactivation by direct and indirect mechanisms. However, it cannot be established from this study whether the effect of the tetracyclines on the level of SL is direct, or indirect through the reduction in microbial challenge which could reduce the microbial proteases and inflammatory mediators such as cytokines, both of which could reduce the levels of human MMP.

After treatment the level of free TIMP increased. This might be due to a reduction in MMPs which would bind to free TIMP. [31-33] The elevation of GCF TIMP was higher in the metronidazole group than in the other groups. This indicates that the regulation of TIMP is not solely dependent on the MMPs. The level of GCF TIMP was significantly elevated in the patients who were treated with antimicrobial agents. However it did not significantly change in the scaling group. The observation of a greater clinical improvement in all three adjunctive antimicrobial treatment groups compared with the scaling and root planing alone supports our biochemical findings and suggests that the extent of

healing in the patients who received local delivery antibiotics was greater than the group who only received scaling and root planing.

It has been suggested that MMP and TIMP may be regulated independently.[34]

In conclusion, adjunctive treatment of locally delivered antibiotics, particularly, tetracycline fibre and minocycline gel, is able to decrease the production of SL in the GCF, although whether this is through direct or indirected mechanisms remains unclear. However the correlation between reduced SL and improved clinical parameters is confirmatory (Harian *et al.* 1995) that SL is a marker of periodontal health.

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Table 1. Mean differences (baseline - after treatment) of biochemical and clinical parameters after therapy in four treatment groups.

Treatment		ΔSL		ΔTIMP		Attachment gain		Pocket depth reduction*	
	n	mean (SEM)	p*	mean (SEM)	p*	mean (SEM)	p*	mean (SEM)	p*
S &TC Fibre	51	0.11 (0.05)	0.020	-0.32 (0.07)	<0.001	0.75 (0.12)	<0.001	1.35 (0.17)	<0.001
S & Min Gel	52	0.13 (0.06)	0.023	-0.22 (0.08)	0.005	0.40 (0.14)	0.013	0.813 (0.13)	<0.001
S & Met Gel	52	0.01 (0.30)	0.730	-0.38 (0.07)	<0.001	0.59 (0.14)	0.001	0.965 (0.18)	<0.001
S	51	-0.01 (0.06)	0.940	+0.05 (0.07)	0.490	0.26 (0.11)	0.032	0.604 (0.14)	<0.001

* The S & TC showed a significantly greater pocket depth reduction than the S (p= 0.003).
* Paired t-test, baseline and post-therapy for each treatment
S & TC Fibre= Scaling and Tetracycline fibre
S & MIN Gel= Scaling and Minocycline gel
S & Met Gel= Scaling and Metronidazole gel
S= Scaling alone

Table 2. Statistical analysis of changes of GCF level of Stromelysin between test groups (n=206).

A. Analysis of covariance					
Source	**DF**	**SS**	**MS**	**F**	**p**
Baseline SL	1	14.59	14.59	291.03	<0.001
Treatments	3	0.897	0.30	5.97	0.001
Error	47	10.07	0.05		
Total	51	25.38			

B. Significance			
	Scaling & Min Gel	**Scaling & Met Gel**	**Scaling alone**
Scaling & TC Fibre	0.658	0.509	0.004
Scaling & Min Gel		0.377	0.002
Scaling & Met Gel			0.035

TC Fibre= Tetracycline fibre
MIN Gel= Minocycline gel
Met Gel= Metronidazole gel
DF= Degree of freedom
SS= sum of square
MS= Mean of square
* Adjusted for baseline level
** After Bonferroni correction the significant threshould was <0.020

Table 3. Statistical analysis of changes of GCF level of TIMP between test groups (n=206).

A. Analysis of covariance					
Source	**DF**	**SS**	**MS**	**F**	**p**
Baseline TIMP	1	20.53	20.53	119.25	<0.001
Treatments	3	2.13	0.71	4.13	0.007
Error	201	34.61	0.17		
Total	205	60.73			

B. Significance			
	Scaling & Min Gel	**Scaling & Met Gel**	**Scaling alone**
Scaling & TC Fibre	0.503	0.170	0.036
Scaling & Min Gel		0.400	0.018
Scaling & Met Gel			0.001

TC Fibre=Tetracycline fibre
Min Gel= Minocycline gel
Met Gel= Metronidazole gel
DF= Degree of freedom
SS= Sum of square
MS= Mean of square
* Adjusted for baseline level
** After Bonferroni correction the significant threshould was <0.020

Table 4. Comparison mean (SEM) of GCF SL and TIMP levels for different gingival inflammation scores at baseline, using Kruskal-wallis test.

	n	**Stromelysin(pg/30s)**	**TIMP(pg/30s)**
MGI=0	6	16.70 (4.12)	295.0 (115)
MGI=1	29	11.50 (1.03)	234.0 (53.2)
MGI=2	112	18.64 (2.28)	326.1 (38.9)
MGI=3	59	28.45 (6.53)	263.4 (38.1)
p value		0.028	0.395

MGI= Modified gingival index