Translational studies to evaluate plaque control interventions

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Abstract

Clinical research should aim to broaden and translate the understanding of health and disease by designing and successfully implementing interventions to achieve healthcare improvement. This thesis reports clinical research that moves from laboratory to clinic and investigates the potential challenges of dissemination and adoption into clinical practice.

Initially an established gingivitis was used as a model to evaluate a personalised plaque control intervention. The evaluation used traditional clinical monitoring techniques and pioneering laboratory technologies. Subsequently the personalised plaque control intervention was developed further and applied to a new clinical situation, the gingival manifestations of oral lichen planus. The personalised plaque control intervention was then evaluated as part of a randomised controlled trial using traditional clinically observed, patient-centred and health-economic outcome measures. Finally, a qualitative study investigated the potential barriers in disseminating research through continuing education to general dental practitioners.

The research findings showed that in the established gingivitis model, sequential plaque control interventions, comprising powered toothbrushing and professional prophylaxis, were effective in reducing the clinical signs of established gingivitis. Changes in clinical signs were associated with a shift in bacterial species, and transient changes were observed in host inflammatory biomarker concentrations. Personalised plaque control was cost-effective and reduced clinical signs of inflammation and brought about improvements in quality of life for patients with gingival manifestations of oral lichen planus. The qualitative study identified barriers to the successful translation and implementation of contemporary clinical research.

The plaque control intervention evaluated in the established gingivitis model and successfully implemented in a new clinical situation. Personalised plaque control should form part of the initial management phase for patients with gingival manifestations of oral lichen planus. Researchers should investigate alternative methods for engaging with general dental practitioners in disseminating research to ensure that relevant findings are translated into improvements in healthcare.

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I would like to express my thanks to my supervisors Professor Peter Heasman and Dr Giles McCracken for their continued support and authoritative guidance over the last five years. I am indebted to them for their inspiration, mentorship and meticulous attention to detail that has enriched this post-graduate experience.

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Clinical studies are impossible without the willingness of patients, staff and dental students to be involved in clinical research. I extend my thanks to all participants of the studies without whom this thesis would be considerably lighter!

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Table of Contents

Index of Tables

Index of Figures

List of Abbreviations

ANOVA	Analysis of variance
BDJ	British Dental Journal
BDS	Bachelor of Dental Surgery
BMA	British Medical Association
BMB	Barcoded Magnetic Beads
BMI	Body Mass Index
BSOM	British Society of Oral Medicine
CAL	Clinical Attachment Loss
CC	Chemotactic Cytokines
CCD	Charged Couple Device
CEJ	Cemento-Enamel Junction
CI	Confidence Interval
cm	Centimetre
COHQOL	Child Oral Health Quality of Life Questionnaire
COMDQ	Chronic Oral Mucosal Disease Questionnaire
CopDend	Committee of Postgraduate Dental Deans and Directors
COREQ	Consolidated Criteria for Reporting Qualitative Research
CPD	Continuing Professional Development
CPITN	Community Periodontal Index of Treatment Need
CQC	Care Quality Commission
CXC	Chemokines
DIDL	Daily Impact on Daily Living
DIP	Dental Impact Profile
DNA	Deoxyribonucleic acid
DoH	Department of Health
ELISA	Enzyme Linked Immunosorbent Assay
EPCRC	European Palliative Care Research Collaborative
ES	Endotoxin Specific
FGDP(UK)	Faculty of General Dental Practitioners (United Kingdom)
FiCTION	Filling Children's Teeth: Indicated or Not?
g	Grams
GCF	Gingival Crevicular Fluid
GCP	Good Clinical Practice
GDC	General Dental Council
GDP	General Dental Practitioner
GDS	General Dental Services
GI	Gingival Index
GOHAI	Geriatric (General) Oral Health Assessment Index
H&E	Haematoxylin and eosin
HAD	Hospital Anxiety and Depression Scale
H_o	Null Hypothesis
HRQOL	Health Related Quality of Life

ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IgA	Immunoglobulin A
IL	Interleukin
Inc	Incorporated
INTERVAL	INTERVAL Dental Recalls Trial
IQUAD	Improving the Quality of Dentistry
Kg	Kilograms
LED	Light Emitting Diode
LS	Least Squares
MAP	Multianalyte Profiling
Max	Maximum
MClin Dent	Master of Clinical Dentistry
MDS	Mucosal Disease Score
Min	Minimum
MIP	Macrophage Inflammatory Protein
MITT	Modified Intention to Treat
ml	Millilitres
MMP	Matrix Metalloproteinase
MSc	Master of Science
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
OHIP	Oral Health Impact Profile
OHIP-14	14 -Item Shortened Oral Health Impact Profile
OHIP-20	20-Item Shortened Oral Health Impact Profile
OHIP-49	49-Item Oral Health Impact Profile
OHRQoL	Oral Health Related Quality of Life
OIDP	Oral Impact on Daily Performance
OLL	Oral Lichenoid Lesion
OLP	Oral Lichen Planus
OMDS	Oro-Mucosal Disease Score
PBS	Phosphate Buffered Saline
PBS-T	Phosphate Buffered Saline Tween
PCR	Polymerase Chain Reaction
PD	Probing Depth
PDP	Personal Development Plan
PE	Phycoerythrin
pg	Picogram
PI	Plaque Index
PPD	Periodontal Probing Depth
PUVA	Psoralen Combined with Ultraviolet A
QALY	Quality Adjusted Life Year
QoL	Quality of Life
QTS	Quadrant, Tooth, Site
R&D	Research and Development
RANTES	Regulated on Activation Normal T-Cell Expressed
REC	Research Ethics Committee

RNA	Ribonucleic Acid
SA-PE	Streptavidin Conjugated Phycoerythrin
SD	Standard Deviation
SE	Standard Error
SF-12	Medical Outcomes Study 12 Item Short Form
SF-36	Medical Outcomes Study 36 Item Short Form
SIGN	Scottish Intercollegiate Guidelines Network
SIP	Sickness Impact Profile
SOOQ	Surgical Orthodontic Outcome Questionnaire
SPCDS	Salaried Primary Care Dental Service
TNF	Tissue Necrosis Factor
UK	United Kingdom
UNC	University of North Carolina
USA	United States of America
VAS	Visual Analogue Scale
VDP	Vocational Dental Practitioner
WHO	World Health Organisation
WTP	Willingness to Pay

Introduction

One of the greatest challenges in contemporary medical and dental research is efficiently utilising scarce resources to translate results into meaningful health benefits for patients, whilst further broadening our understanding of the basic processes that underlie states of both health and disease. The traditional surrogate outcome measures that are so often applied to controlled clinical trials are very much clinician centred, despite the growing acceptance of qualitative research within medicine. In some studies, when patient-centred outcome measures do not show the intended outcome, claims are made that the instrument itself is at fault and it may not be appropriately measuring the correct construct.

Some fields of medical research use the most finite of endpoints, life and death. The majority of chronic debilitating disorders, however, have recognisable symptoms and measurable signs that can be managed. These finite outcome measures are therefore not appropriate. In dentistry, perhaps the absolute finite outcome measure is tooth loss, and whilst this could be appropriate with the more common oral diseases of caries and periodontal disease, it cannot be used for soft tissue pathologies (other than cancer). The ethical responsibilities of healthcare professionals ensure that cost effective and efficacious interventions are recommended and delivered before these finite endpoints are reached. Identifying predictors of future disease, therefore, has been the focus of contemporary oral health research. Interventions have been designed to not only resolve pathologies but also to help understand the underlying disease mechanisms and aetiologies. The demand on finite resources also means that studies that are funded and approved should maximise the potential for gaining valuable information on the disease or intervention being examined.

Ultimately, research must also be accessible to those that can most easily implement improvements in practice. In dentistry this lies outside of research-intensive institutions in general dental practice. As such researchers must understand the challenges and pressures that are placed on practitioners so that they might engage positively together to bring about change.

Oral health interventions are recommended by general dental practitioners, industry and through the media every day. Tailoring an intervention to an individual patient requires a deeper understanding of the disease being treated, how the intervention affects the underlying biology, and how it will bring about improvements in oral health and, even, potentially quality of life.

This thesis describes the evaluation of such an intervention, exploring the underlying pathologies and examining the effect it has from the patients' perspective. It also attempts to distil down some of the problems that general dental practitioners face in making meaningful and cost-effective changes to their practice.

Chapter 1

Literature Review

Prologue

This literature review is focussed upon the areas that are pertinent to the development and translation of the investigations described within this thesis. It will examine the aetiology of gingival manifestations of oro-mucosal disease, with specific reference to oral lichen planus. The role of patient-centred outcome measures, biomarkers, and health economics will be discussed along with the relevance, importance, and practicalities of their use within clinical trials. Clinical research must be accessible and relevant for it to be successfully translated into practice. The potential challenges and barriers that may prevent this from occurring are presented.

1.1 Lichen planus

Lichen planus is a chronic autoimmune inflammatory disease with T-cell mediated immunological dysfunction. The aetiology of the disease is poorly understood. Links have been hypothesised between lichen planus and the local environment, high stress levels, viruses and abnormal host response, but there is little evidence to categorically support or dismiss any of these claims (Koray *et al.*, 2003; Sun *et al.*, 2005; Annes and Szepietowski, 2007; Lodi *et al.*, 2010). Lichen planus is often symptomatic, however current available treatments are not curative and are aimed at controlling painful symptoms to comfortable levels (Thornhill, 2001; Lodi *et al.*, 2005a; Thongprasom *et al.*, 2011).

Lichen planus may affect multiple sites including the skin, oral and nasal mucosa, ears, eyes, as well as the gastrointestinal tract and the genitalia (Neumann *et al.*, 1993; Eisen, 1999; Abraham *et al.*, 2000; Cheng *et al.*, 2012). The most common cutaneous presentations are on the flexural surfaces of wrists or ankles and appear as purple plaques or raised areas. The surface of these lesions may have a lacy white appearance known as Whickham's striae (Figure 1) (Boyd and Nelder, 1991; Breathnach and Black, 2004; Cheng *et al.*, 2012). The skin symptomatology is that of itchiness, and in contrast to some of the more erosive presentations that often affect mucosal surface, skin lesions have the best prognosis with many resolving

with treatment within 2 years (Boyd and Nelder, 1991; Breathnach and Black, 2004).

1.1.1 Oral lichen planus

The majority of patients with cutaneous lesions (up to 70%) also have oral involvement. The oral presentations vary from mild keratosis to extensive erosions and ulceration (Figure 2) (Altman and Perry, 1961; Samman, 1961; Leao *et al.*, 2008; Lo Russo *et al.*, 2008). The prevalence of oral lichen planus is between 0.5% and 2.0% of studied populations with the most recent estimation being 1.27% (Thongprasom *et al.*, 2011). The distribution of oral lichen planus in the population shows an increased prevalence in females with a higher percentage of women between 30 and 60 years of age being affected (McCartan and Healy, 2008; Thongprasom *et al.*, 2011). The terms commonly used to describe the appearance of oral lichen planus lesions are: reticular, papular, desquamative, erosive (ulcerative), atrophic, bullous, plaque-like and combination forms (Andreasen, 1968; Eisen, 2002; Lodi *et al.*, 2005a; Escudier *et al.*, 2007; Thongprasom *et al.*, 2011). The most common and least symptomatic oral presentation is that of reticular lichen planus which occurs symmetrically on the buccal mucosa but may also present on the gingivae, tongue, palate and lips (Kramer *et al.*, 1978; Lozada-Nur and Miranda, 1997; Thongprasom *et al.*, 2011). The only presenting complaints of patients with milder presentations may be sensitivity to spicy or acidic foods. The more widespread atrophic, erosive and ulcerative presentations of the disease are much more likely to be symptomatic and have a significant impact on patients' lives (Cheng *et al.*, 2012).

Those with erosive oral lesions may also have genital involvement. This more severe presentation in women is known as the vulvovaginal-gingival syndrome, and in men the peno-gingival syndrome (Pelisse *et al.*, 1982; Pelisse, 1989; Cribier *et al.*, 1993). The various sites affected by oral lichen planus may lead to presentation in different healthcare settings including general practice, specialist oral medicine, dermatology, genitourinary or gastroenterology clinics (Cheng *et al.*, 2012). There may be the need for multidisciplinary management or joint specialist consultations in the cases where multiple sites are affected to ensure appropriate diagnosis and management.

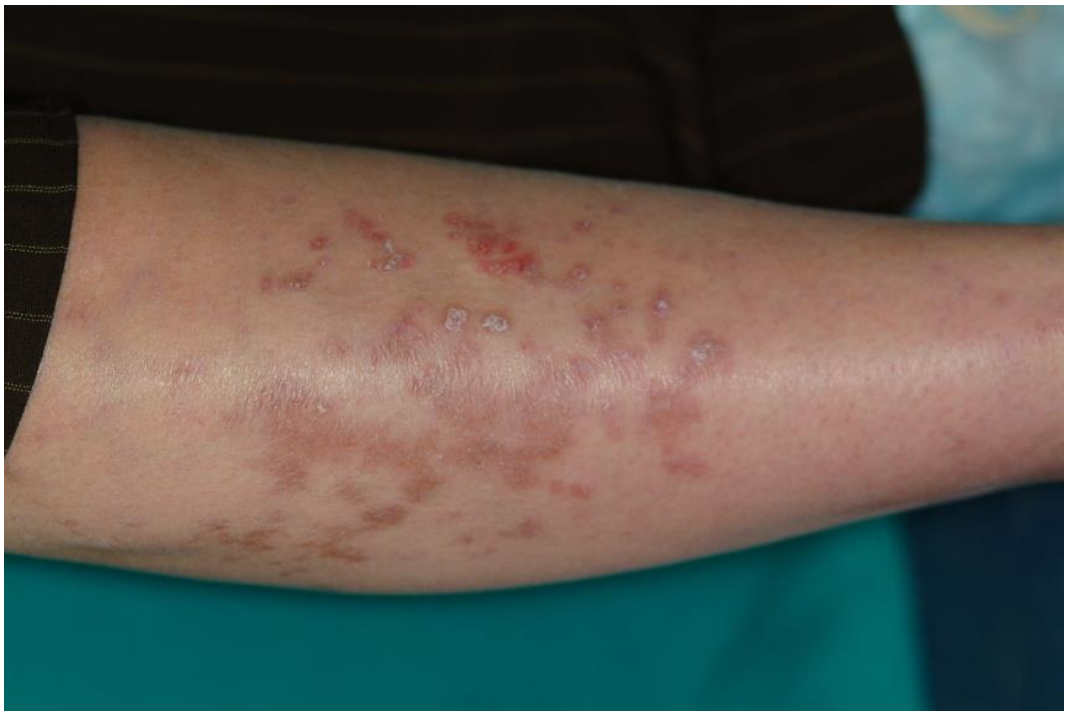


Figure 1. A cutaneous presentation of oral lichen planus.

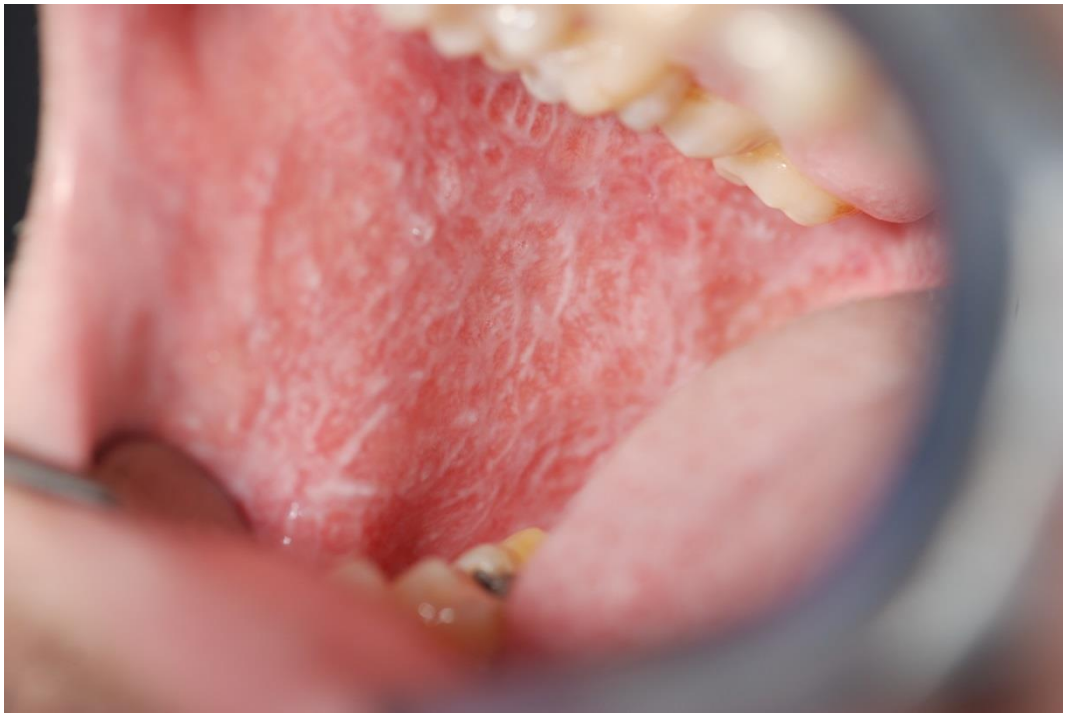


Figure 2. A classical reticular presentation of oral lichen planus.

Diagnosis can usually be made on the clinical presentation alone although biopsy and histopathological examination are seen as the gold standard to eliminate the diagnoses of vesiculobullous disorders, dysplasia or malignancy (Eisen *et al.*, 2005; Scully and Carrozzo, 2008; BSOM, 2010). Although there is some controversy over whether all cases of suspected oral lichen planus are biopsied, cases that have an atypical presentation, are atrophic or ulcerative should be biopsied (BSOM, 2010).

There are characteristic histopathological features in oral lichen planus (Figure 3). Care must be taken in interpreting the microscopic appearance by relating it to the normal structure for the area from which it was taken.

Haematoxylin and Eosin stained samples of affected oral mucosa clearly show characteristic rete processes with a 'saw tooth' like appearance and epithelial atrophy may be also seen (Figure 3) . The basal cell layer will usually include signs of liquefactive degeneration with a superficial band of T-lymphocytes present in the connective tissue (Kramer *et al.*, 1978; Eisenberg, 2000; Thongprasom *et al.*, 2011).

There is a large but inconsistent body of evidence to suggest that oral lichen planus may be a pre-malignant condition. In 1978 the World Health Organisation (WHO) proposed that oral lichen planus has a predisposition to malignant change (Kramer *et al.*, 1978). The most recent observations found that the frequency of transformation into squamous cell carcinoma ranged between 0% to 12.5%, whereas previous data suggest that the rate of transformation into squamous cell carcinoma is much lower, between 0.5% and 5% (Silverman *et al.*, 1985; Holmstrup *et al.*, 1988; Lo Muzio *et al.*, 1998; Gandolfo *et al.*, 2004; Rodstrom *et al.*, 2004; Hsue *et al.*, 2007; Gonzalez-Moles *et al.*, 2008). The problem with identifying a precise transformation rate lies with the differing definitions of oral lichen planus, in particular whether all presentations were included in the studies along with the size of the populations observed. The transformation rate is further skewed by the difficulty in differentiating between true malignant transformation and a carcinoma occurring in patients with oral lichen planus who also are tobacco and alcohol users and, therefore, inherently have a higher risk for oral malignancy (Gonzalez-Moles *et al.*, 2008). There is some evidence to suggest that patients with erosive and atrophic presentations along with these risk factors will have a higher risk of malignant transformation (Scully and Carrozzo, 2008).

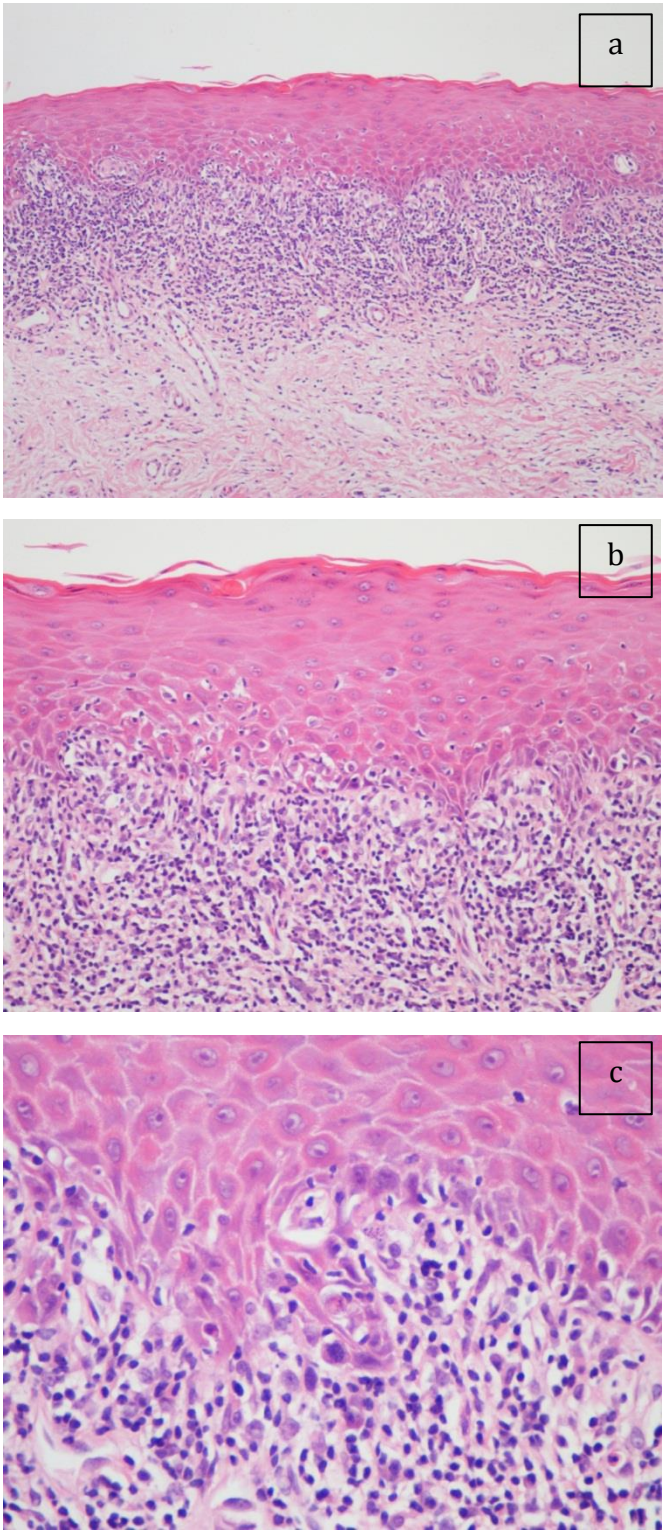


Figure 3. Haematoxylin eosin (H&E) staining of incisional biopsy of oral lichen planus indicating sub-epithelial lymphocytic infiltration, 'saw like' rete processes and disorder at the basement membrane at x10 (a), x20 (b) and x30 (c) magnifications.

It is for this reason that patients with oral lichen planus are reviewed routinely and there is a low threshold for further biopsy if the clinical features of the lesions worsen (Gonzalez-Moles *et al.*, 2008).

1.1.2 Oral lichenoid lesions

Oral lichenoid lesions (OLL) closely mimic those of oral lichen planus in both their clinical and histological appearance. The aetiology is reactionary in nature and is widely attributed to dental amalgam and a number of medications. These drugs include beta-blockers, thiazide diuretics, angiotensin converting enzyme inhibitors, calcium channel blockers, sulphonylureas, anti-malarials, gold, penicillamine, allopurinol and non-steroidal anti-inflammatory agents (Chau *et al.*, 1984; Potts *et al.*, 1987; Firth and Reade, 1989; Thornhill *et al.*, 2006; BSOM, 2010). A comprehensive medical history and clinical examination is essential in formulating a diagnosis and differentiating oral lichen planus from that of an oral lichenoid lesion. Even following clinical examination, biopsy and histopathological analysis, the final diagnosis may lie somewhere between oral lichen planus and an oral lichenoid lesion.

1.1.3 Gingival manifestations of oral lichen planus

Desquamative gingivitis is a frequently used descriptive term for the gingival manifestations of oral lichen planus comprising chronic epithelial desquamation, erythema, erosion and blistering of the attached and marginal gingiva (Prinz, 1932; Guiglia *et al.*, 2007). The extent of the desquamation varies from mild localised patches to widespread intense erythema, ulceration and areas of spontaneous haemorrhage (Figure 4) (Scully and Porter, 1997). Originally described as a 'chronic desquamative gingivitis' and later as a 'chronic diffuse desquamative gingivitis,' the aetiology was initially considered to be either idiopathic or related to hormone changes around menopause in middle aged females (Tomes and Tomes, 1894; Prinz, 1932). It is now attributed to a number of autoimmune conditions as well as adverse reactions to a variety of pharmaceuticals, chemicals and allergens (Scully and Porter, 1997; Stoopler *et al.*, 2003; Leao *et al.*, 2008; Lo Russo *et al.*, 2009).

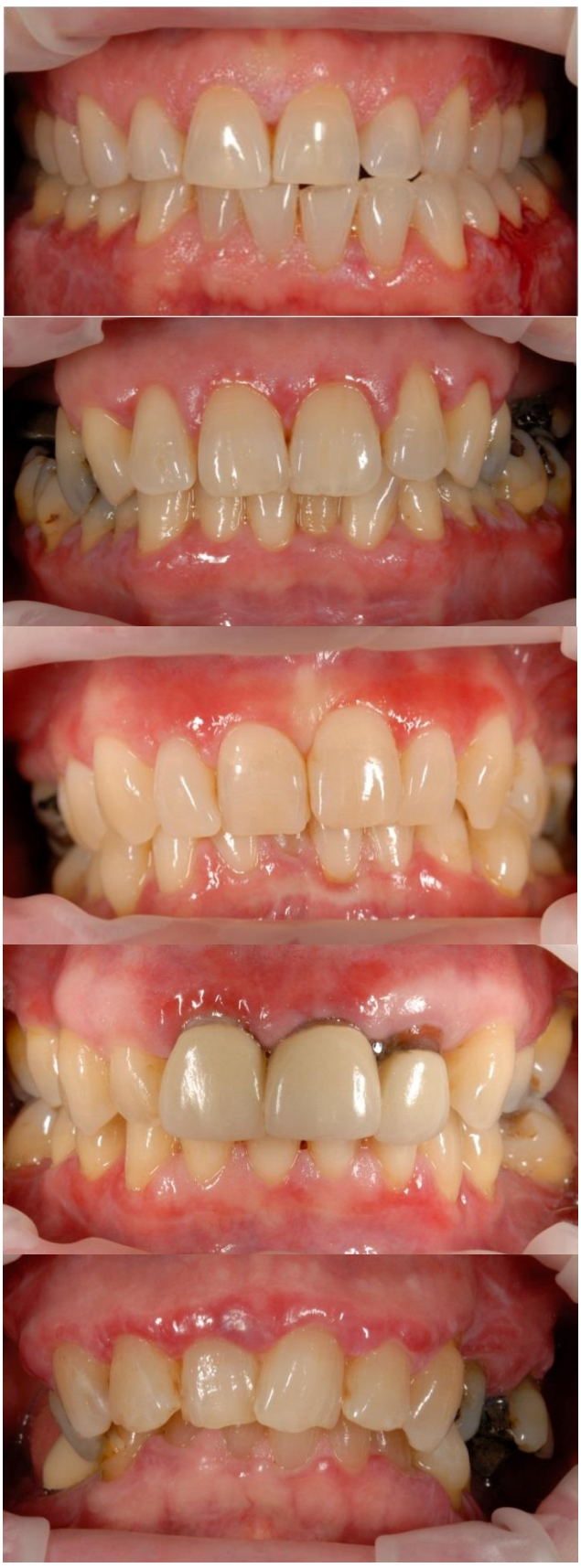


Figure 4. Presentations of the gingival manifestations of oral lichen planus (from participants in Clinical Trial 2 in this thesis).
The clinical appearance varies from mild keratosis and classical Whickham's striae (a) to localised (c) and widespread erythema and glazing (d, e). Localised areas of ulceration (a, b, c, d) and spontaneous haemorrhage (a) are also shown.

Desquamative gingivitis has been linked or associated with:

- oral lichen planus;
- oral lichenoid lesions;
- mucous membrane pemphigoid;
- pemphigus vulgaris, erythema multiforme;
- graft versus host disease;
- lupus erythematosus;
- paraneoplastic pemphigus;
- epidermolysis bullosa acquitista;
- linear immunoglobulin A (IgA) disease;
- ulcerative stomatitis;
- plasma call gingivitis;
- dermatitis herpetiformis;
- foreign body gingivitis;
- psoriasis.

Many of these conditions are rare and most commonly, oral lichen planus is the underlying cause of a desquamative gingivitis (Lo Russo *et al.*, 2009). It is seen most commonly in the erosive, ulcerative and atrophic forms and may be the only sign of oral involvement (Jadinski and Shklar, 1976; Scully and Porter, 1997).

1.1.2 Treatment of oral lichen planus

Treatment is normally initiated to manage the pain and severity of the symptoms. Current evidence and guidance suggests that topical corticosteroids should be the first line treatment. There is however no universally agreed second line treatment but short courses of systemic corticosteroids have been suggested (Cribier *et al.*, 1998; Carrozzo and Gandolfo, 1999; Eisen, 2002; Eisen *et al.*, 2005; Lodi *et al.*, 2005b; Al-Hashimi *et al.*, 2007; Scully and Carrozzo, 2008; Carrozzo and Thorpe, 2009; BSOM, 2010; Cheng *et al.*, 2012). The current pathway outlining the recommendations for the clinical management of symptomatic oral lichen planus is outlined in Figure 5 (Lodi *et al.*, 2005b).

Despite the widespread acceptance and use of topical corticosteroids in the treatment of symptomatic oral lichen planus, there are currently no randomised controlled trials that investigate their effectiveness against a placebo (Lodi *et al.*, 2012). Studies have compared topical corticosteroids of varying potencies and have included fluticasone propionate, betamethasone sodium phosphate, prednisolone, clobetasol propionate and triamcinolone.

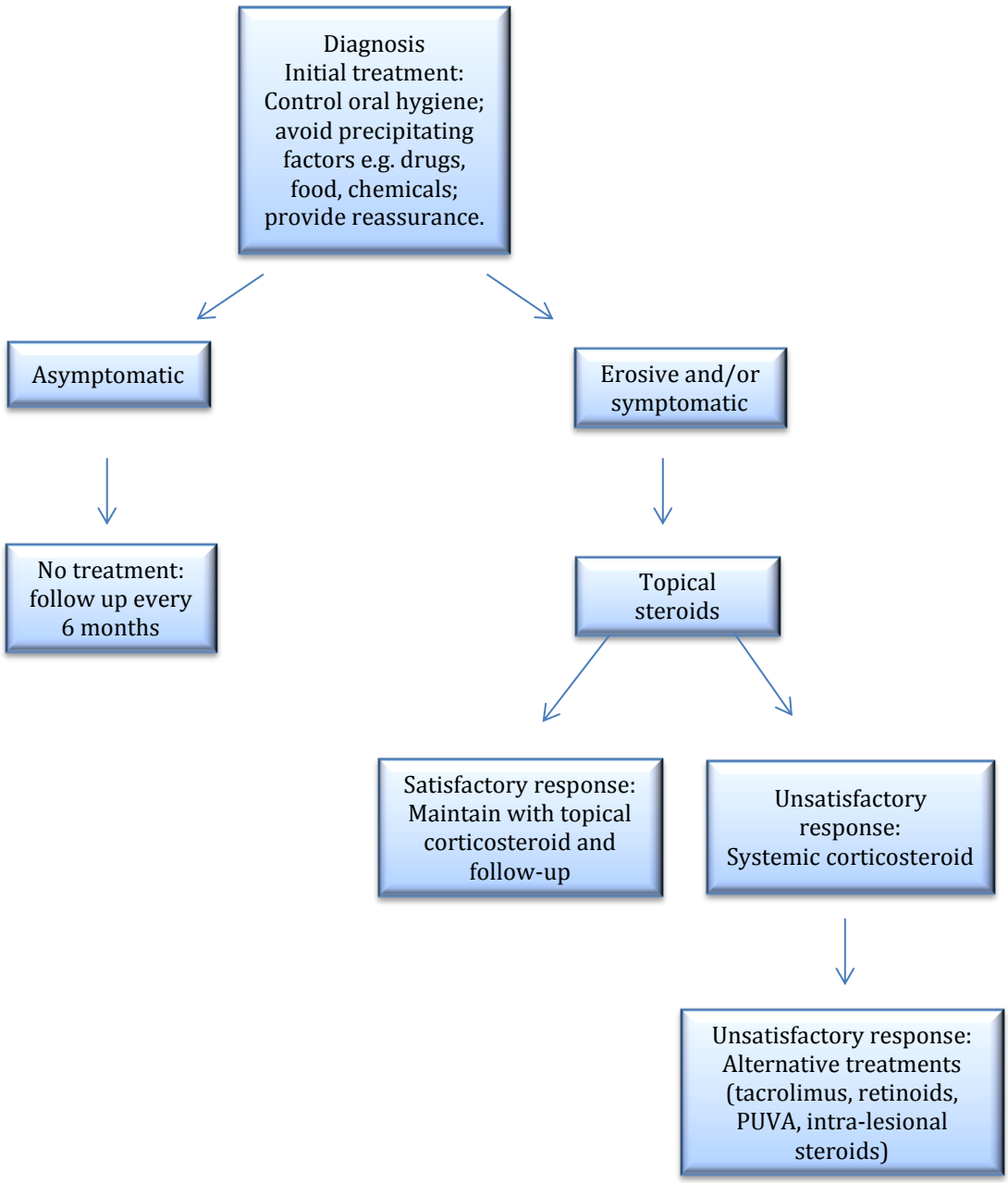


Figure 5. An overview of the clinical management of oral lichen planus (Lodi *et al.*, 2005a).

The majority of trials have recruited patients with erosive lichen planus and effectiveness of one preparation over another remains inconclusive (Rödström *et al.*, 1994; Hegarty *et al.*, 2002a; Campisi *et al.*, 2004; Malhotra *et al.*, 2008; Carbone *et al.*, 2009; Ghabanchi *et al.*, 2009; Lodi *et al.*, 2012).

Topical calcineurin inhibitors have been widely investigated and compared with the effectiveness of topical corticosteroids. Of these, the most commonly used are ciclosporin, tacrolimus and pimecrolimus. Evidence suggests that there is little difference in the effectiveness of these drugs against topical corticosteroids (Eisen *et al.*, 1990; Conrotto *et al.*, 2006; Yoke *et al.*, 2006; Gorouhi *et al.*, 2007; Passeron *et al.*, 2007; Volz *et al.*, 2008). Their use may be less frequent due to the need to monitor blood biochemistry (electrolytes, blood urea, creatinine, magnesium, lipids, liver function tests and uric acid) during the first few weeks of treatment (Lebwohl *et al.*, 1998).

In comparison, topical corticosteroids are generally well tolerated and have less systemic toxicity than their systemic counterparts (such as adrenocortical insufficiency) (Gonzalez-Moles *et al.*, 2002; Lodi *et al.*, 2012; Varoni *et al.*, 2012). Other topical treatments for symptomatic lesions have included topical anaesthetics (lidocaine gel 2%; benzydamine hydrochloride 0.15% mouthrinse) or barrier agents such as carmellose sodium protective paste (Orabase®).

The outcome of the two most recent systematic reviews of interventions for treating oral lichen planus has recommended that future studies should be designed to better allow meta-analysis of data (Zakrzewska *et al.*, 2005; Lodi *et al.*, 2012). Although not recommending which specific standardised outcome measures should be used, they recommend that all adverse events of treatment are reported and some evaluation of cost-effectiveness should also take place (Zakrzewska *et al.*, 2005; Lodi *et al.*, 2012). The studies included in the review evaluated pharmacological treatment whilst conservative management strategies, for example, were not included (Table 1).

Despite the lack of credence given to conservative strategies in the systematic reviews, factors that have been found to expedite improvement of symptomatic lesions include: reassurance; avoidance of exacerbating factors such as certain foods; avoidance of smoking and alcohol; and improving oral hygiene (Ramon-

Fluixa *et al.*, 1999; Thongprasom *et al.*, 2003; Thongprasom *et al.*, 2011). There are currently no randomised controlled trials investigating the effectiveness of more conservative, non-pharmacological interventions.

Intervention	Author	Number of participants	Outcome measure	Follow up period
Ciclosporin rinse 100 $mgml^{-1}$ vs placebo	Eisen *et al.*, 1990	16	Pain (4-point scale), erosion and erythema (4-point scale)	8 weeks
Ciclosporin 16mg gel vs placebo	Gombos *et al.*, 1992	20	Complete healing, reduction in clinical signs of oral lichen planus	10 weeks
Flucinonide 0.025% vs placebo	Voute *et al.*, 1993	40	Pain (5-point scale), clinical score (5-point scale)	9 weeks
Clobetasol propionate vs triamcinolone	Rödström *et al.*, 1994	40	Pain (Visual Analogue Scale), clinical score (4-point score)	9 weeks
Photochemotherapy with 8-methoxypsoralen (0.6 $mgkg^{1}$) and long-wave ultraviolet light vs no treatment	Lundquist *et al.*, 1995	18	Clinical score (3-point scale)	12 months
Fluticasone propionate 50µg spray vs betamethasone sodium phosphate 500µg mouthwash	Hegarty *et al.*, 2002a	39	Pain (Visual Analogue Scale), clinical score (area mm^2 lesions), quality of life (OHIP, OHQoL)	6 weeks
Traditional Chinese medicine (herbal medicine plus prednisolone 5-10mg plus chlorphenamine 4 mg plus vitamin C) vs western drugs (prednisolone 5-10 mg, chlorphenamine 4mg plus vitamin C)	Xu *et al.*, 2002	39	Clinical score (4-point scale), relapse rate	6 weeks
Mycostatin (nystatin) paste and dexamethasone paste	Wei *et al.*, 2003	57	Clinical score (4-point scale)	6 weeks
Comparing 2 formulations of clobetasol propionate 0.025% (lipid-loaded microspheres & lipophilic ointment in a hydrophilic phase)	Campisi *et al.*, 2004	45	Pain (Visual Analogue Scale), Clinical score (Thongprasom *et al.*, 1992)	2 months
1% pimecrolimus cream vs placebo	Swift *et al.*, 2005	20	Pain (Visual Analogue Scale), clinical score (weighted area)	4 weeks

Clobetasol propionate 0.025% vs ciclosporin 1.5%	Conrotto *et al.*, 2006	39	Pain (Visual Analogue Scale), clinical score (Thongprasom *et al.*, 1992), cost	2 months
Tacrolimus 0.1% ointment vs triamcinolone acetonide 0.1%	Laeijendecker *et al.*, 2006	40	Clinical score (4-point scale)	6 weeks
Ciclosporin solution 0.1% vs triamcinolone acetonide 0.1% in orabase	Yoke *et al.*, 2006	139	Pain (Visual Analogue Scale), clinical score (Thongprasom *et al.*, 1992)	8 weeks
Curcuminoids 2000mg and prednisolone 60mg vs prednisolone 60mg plus placebo	Chainani-Wu *et al.*, 2007	33	Pain (Visual Analogue Scale), clinical score (modified oral mucositis index), global change scale,	7 weeks
Pimecrolimus 1% cream vs triamcinolone acetonide 0.1%	Gorouhi *et al.*, 2007	40	Pain (Visual Analogue Scale), clinical score (Thongprasom *et al.*, 1992), quality of life (OHIP)	2 months
Clobetasol propionate 0.05% with adjunctive miconazole 2% vs clobetasol propionate 0.05% with placebo	Lodi *et al.*, 2007	35	Pain (Visual Analogue Scale), clinical score (percentage of mucosa affected)	6 weeks
1% pimecrolimus cream vs placebo	Passeron *et al.*, 2007	12	Pain (5 point score), clinical score (erosive area)	4 weeks
Aloe vera 70% vs placebo	Choonhakarn *et al.*, 2008	54	Pain (Visual Analogue Scale), clinical response (4-point scale)	8 weeks
Tacrolimus 0.1% ointment vs clobetasol propionate 0.05%	Corrocher *et al.*, 2008	32	Pain (4-point scale), clinical score (4-point scale)	4 weeks
Betamethasone oral mini-pulse therapy vs triamcinolone acetonide 0.1%	Malhotra *et al.*, 2008	49	Pain, clinical score	6 months
1% pimecrolimus cream vs placebo	Volz *et al.*, 2008	20	Pain (Visual Analogue Scale), clinical score (composite score)	30 days
Clobetasol propionate 0.025 vs clobetasol propionate 0.05%	Carbone *et al.*, 2009	35	Pain (Visual Analogue Scale), clinical score	2 months

Triamcinolone acetonide 0.1% vs prednisolone 5mg mucoadhesive tablet	Ghabanchi *et al.*, 2009	20	Clinical score (5-point scale)	2 weeks
Ignatia 30c (homeopathic medicine)	Mousavi *et al.*, 2009	30	Pain (Visual Analogue Scale), clinical score (semi-quantitative scoring system)	4 months
Hyaluronic acid vs placebo	Nolan *et al.*, 2009	124	Clinical score (erosive area), functional score (eating)	28 days
Intralesional injection 0.5ml BCG-PSN vs 10mg triamcinolone acetonide 0.25ml from solution 40 $mgml^{-1}$ mixed 0.25ml 2% lidocaine for injection	Xiong *et al.*, 2009	56	Pain (Visual Analogue Scale), clinical score (erosive area)	3 months
Pursulane 235mg (herbal medicine) vs placebo	Agha-Hosseini *et al.*, 2010	37	Pain (Visual Analogue Scale), clinical score (percentage of mucosa affected)	3 months
Aloe vera vs placebo	Salazar-Sanchez *et al.*, 2010	55	Pain (Visual Analogue Scale), clinical score (Thongprasom *et al.*, 1992), treatment response, quality of life (OHIP-49), hospital anxiety and depression scale (Snaith, 2003))	12 weeks

Table 1. Interventions for the management of oral lichen planus, adapted from a Cochrane systematic review (Thongprasom *et al.*, 2011; Lodi *et al.*, 2012).

A painful ulcerative or erosive desquamative gingivitis has the potential to interfere with effective implementation of daily oral hygiene procedures (Lo Russo *et al.*, 2009). This may lead to the accumulation of dental plaque, which has been reported to induce or worsen the activity of the lesions especially in the case of oral lichen planus (Holmstrup *et al.*, 1990; Lo Russo *et al.*, 2008). The importance of mechanical and self-performed plaque control in patients with periodontal disease and in patients undergoing periodontal maintenance is well documented (Ramfjord *et al.*, 1982; Rosling, 1983; Ramfjord, 1987; Rosen *et al.*, 1999; Allen *et al.*, 2008). The reported effect of dental plaque on the gingival manifestations of oral lichen planus, suggests that whilst good oral hygiene may not bring about complete resolution, the presence of plaque and calculus deposits irritates, aggravates and exacerbates the disease (Erpenstein, 1985; Holmstrup *et al.*, 1990; Guiglia *et al.*, 2007). Although poor plaque control is likely to compromise periodontal health, little evidence exists to suggest that patients with gingival manifestations of oral lichen planus generally have poorer oral hygiene or exhibit greater or more frequent periodontal attachment loss (Ramfjord *et al.*, 1982; Rosling, 1983; Ramfjord, 1987; Rosen *et al.*, 1999; Allen *et al.*, 2008; Lo Russo *et al.*, 2008). The optimisation of plaque control may prevent periodontal damage and reduce symptoms associated with the gingival manifestations of oral lichen planus despite the current limited evidence and lack of well-designed trials (Lo Russo *et al.*, 2008).

Despite this weak evidence, most guidelines and reviews have recommended that as part of the initial treatment, oral hygiene should be improved and controlled. Improvements in oral hygiene may convey a benefit in reducing the frequency and severity of symptomatic oral lichen planus but other factors may confound the strength of this evidence (Erpenstein, 1985; Holmstrup *et al.*, 1990; Guiglia *et al.*, 2007; Lopez-Jornet and Camacho-Alonso, 2010a).

Holmstrup et al. (1990) followed a small cohort of 11 patients with oral lichen planus-attributed desquamative gingivitis who were treated with an 'intensive individual hygiene programme' with 'frequent professional assistance over one year'. This assistance included advice on the use of toothbrushes, toothsticks, floss, chlorhexidine 0.2% mouthwash and hibitane toothpaste (1% chlorhexidine) (Holmstrup *et al.*, 1990). In addition to receiving this regimen of hygiene phase

therapy, patients also underwent treatment of caries and renewal of deficient restorations. The authors do not discuss the materials used or if amalgam restorations were replaced with alternatives. They measured subjective symptoms by using a global change scale. Whilst this study noted improvements in all of the patients' symptoms, the study cohort was small and the subjective outcome measure used was not previously tested for validity or reliability. These confounding factors and the size of this study make drawing firm associations between the improvement of poor plaque control and symptomatic gingival lesions difficult.

Guiglia et al. (2007) conducted an open-label, single-blinded study of 30 subjects with gingival manifestations of oral lichen planus over a 3-month period. The aim was to evaluate a combined regimen of oral hygiene and corticosteroid therapy. As part of this, subjects underwent supra and subgingival scaling (if necessary) as well as 30 minutes of oral hygiene advice with soft manual toothbrushes, appropriate interdental cleaning aids, and chlorhexidine. As well as instructions to exercise meticulous, self-performed plaque control they underwent concurrent pharmacological treatment with topical clobetasol propionate, a potent topical corticosteriod (Guiglia *et al.*, 2007). Furthermore, there was no standardisation of how many times per day the subjects were asked to apply the clobetasol ointment (5 minutes once, twice or three times daily). Clinical improvement was assessed using the Silness and Löe plaque index and bleeding on probing. Clinical photographs were also used and two examiners were asked to grade improvement on a yes/no dichotomous scale. The paper did not indicate if the examiners were calibrated for any of the outcome measures; stating that they were trained in 'periodontology.' There are flaws within the design that make it difficult to ascertain how much of the improvement noted in plaque score and bleeding was due to one particular mechanical intervention; either the professional intervention of scaling and polishing or the oral hygiene carried out over the duration of the study. There may also have been benefit from the use of topical steroids, either directly or indirectly, in reducing the inflammation to allow self-performed plaque control. The study did not account for subjects' symptoms and the change in severity of the clinical signs was not robustly recorded (Guiglia *et al.*, 2007).

The most recent study to examine the relationship between the gingival manifestations of oral lichen planus and plaque control examined 40 patients who were provided with a combined protocol of a 'motivation-behavioural skills' for improving oral hygiene and topical corticosteroids (Lopez-Jornet and Camacho-Alonso, 2010a). The design was a pre- and post-test descriptive study with no control group and a follow-up period of 4 and 8 weeks. As with the study by Guiglia et al. (2007) the subjects did not receive any topical steroid treatment for three months prior to their involvement in the study. The outcome measures were all related directly to gingival health and plaque control: Gingival Index (Löe and Silness, 1967); the modified Quigley and Hein Plaque Index (Turesky *et al.*, 1970); and the Community Index of Periodontal Treatment Need (CPITN) (WHO, 1987). The interventions were scaling and polishing, oral hygiene instruction based on the Bass method of tooth brushing and use of topical triamcinolone mouthrinse 0.1% for 1 minute, three times daily. There was no mention of any interdental cleaning aids or the type of toothbrush recommended (Lopez-Jornet and Camacho-Alonso, 2010a). The focus was on the effectiveness of the advice provided and self-care performed by the subjects upon plaque scores and gingival health. Whilst gingival health improved and plaque levels decreased, the study did not identify what were the most important factors between gingival lichen planus and suboptimal plaque control. For example, was the concomitant use of topical corticosteroids the most important factor to facilitate improved plaque control or was it the effectiveness of the motivational-behavioural skills protocol? The focus of the previous studies was centered on change in plaque and not the effect that plaque control has on the severity or activity of oral mucosal disease or symptoms.

The studies that have looked at conservative treatment strategies for oral lichen planus have used traditional approaches to measure oral disease through the use of clinical parameters and indices such as plaque indices, gingival indices, probing depths and attachment levels (Silness and Löe, 1964; Löe and Silness, 1967; Turesky *et al.*, 1970; WHO, 1987). These purely clinical measures may underestimate the effect that the disease has on a patient (Allen *et al.*, 1999). The symptoms of redness, bleeding on brushing, loosening of affected teeth, and persistent bad breath are highly relevant from the patient's perspective (Ng and Leung, 2006) and have the potential to impact adversely on quality of life (Locker, 1988; Aslund *et al.*, 2008).

Where clinical indices are used they should be valid, reliable and reproducible and be able to record a small but clinically important change (Lodi *et al.*, 2012). Various scoring systems have been proposed to monitor the extent of oral lichen planus, but a uniform, standardised approach is not always taken (Sloberg *et al.*, 1983; Eisen *et al.*, 1990; Silverman *et al.*, 1991; Bagan-Sebastian *et al.*, 1992; Thongprasom *et al.*, 1992; Harpenau *et al.*, 1995; Buajeeb *et al.*, 1997; Kaliakatsou *et al.*, 2002; Rozycki *et al.*, 2002; Bethke and Reichart, 2005; Piboonniyom *et al.*, 2005; Escudier *et al.*, 2007; Leao *et al.*, 2008). Most of the interventional trials and studies investigating the effectiveness of treatment of oral lichen planus have relatively simple outcome measures. The efficacy for conservative, topical or systemic management for oral lichen planus currently carries little objective evidence (Escudier *et al.*, 2007). Outcome measures have therefore focused upon simple subjective scores of pain and disease activity (Lodi *et al.*, 2012).

The most frequently encountered scoring system for reporting disease activity in oral lichen planus research was originally reported in a clinical trial of two topical corticosteroids: triamcinolone acetonide 0.1% and fluocinolone acetonide 0.1% (Thongprasom *et al.*, 1992). It recorded disease activity on a 6-point scale relating to the severity of the lesion and the area of erosion or erythema present and the scoring was undertaken by two clinicians (Table 2) (Thongprasom *et al.*, 1992). It has been used in a number of studies as an outcome for comparing the effectiveness of different topical treatments (Campisi *et al.*, 2004; Swift *et al.*, 2005; Conrotto *et al.*, 2006; Yoke *et al.*, 2006; Gorouhi *et al.*, 2007; Salazar-Sanchez *et al.*, 2010).

Despite proposing this now widely adopted scoring system, few data have been presented or any analysis carried out on its validity or reproducibility in the original or subsequent papers (Thongprasom *et al.*, 1992; Campisi *et al.*, 2004; Conrotto *et al.*, 2006; Gorouhi *et al.*, 2007; Carbone *et al.*, 2009; Salazar-Sanchez *et al.*, 2010).

Score	Description
0	No lesion, normal mucosa.
1	Mild white striae, no erythematous areas.
2	White striae with atrophic area less than $1cm^2$.
3	White striae with atrophic area more than $1cm^2$.
4	White striae with erosive area less than $1cm^2$.
5	White striae with erosive area more than $1cm^2$.

Table 2. Shows a scoring system frequently used as an outcome measure in oral lichen planus research (Thongprasom *et al.*, 1992).

In the majority of clinical studies the primary outcome measure used accounts for the patient's symptoms by simple pain scales such as the visual analogue scale (VAS) (Lundquist *et al.*, 1995; Hegarty *et al.*, 2002a; Campisi *et al.*, 2004; Swift *et al.*, 2005; Conrotto *et al.*, 2006; Yoke *et al.*, 2006; Chainani-Wu *et al.*, 2007; Cheretakis *et al.*, 2007; Gorouhi *et al.*, 2007; Lodi *et al.*, 2007; Choonhakarn *et al.*, 2008; Volz *et al.*, 2008; Carbone *et al.*, 2009; Mousavi *et al.*, 2009; Agha-Hosseini *et al.*, 2010; Salazar-Sanchez *et al.*, 2010).

VAS scales are usually administered as a 100mm straight line with the anchors being 'no pain at all' and 'worst imaginable pain;' respondents are asked to place a mark on the line that best fits with their current experience of pain (Hjermstad *et al.*, 2011). These are one-dimensional scales that are generally straightforward to administer. The VAS was originally used to record pain intensity in cancer patients but has subsequently been used in many different aspects of healthcare (Seymour, 1982; Breivik *et al.*, 2000; Jensen, 2003; Caraceni *et al.*, 2005; Hjermstad *et al.*, 2011). Since pain is a frequently encountered symptom by patients with oral lichen planus, VAS scales have been extensively used as an outcome measure. When considered for use in a new study, they should be administered in a standardised manner so that comparisons can be made between patients but more importantly between interventions (Hegarty *et al.*, 2002a; Campisi *et al.*, 2004; Swift *et al.*, 2005; Conrotto *et al.*, 2006; Chainani-Wu *et al.*, 2007; Gorouhi *et al.*, 2007; Lodi *et al.*, 2007; Choonhakarn *et al.*, 2008; Volz *et al.*, 2008; Carbone *et al.*, 2009; Mousavi *et al.*, 2009; Salazar-Sanchez *et al.*, 2010; Lodi *et al.*, 2012). Although VAS scales are the most frequently encountered method of recording pain, other subjective assessments of pain exist such as numerical rating scales and verbal rating scales. More complex, validated instruments have also been developed with which to assess pain. These include the full and short form versions of the McGill Pain Questionnaire, the Brief Pain Inventory and the European Palliative Care Research Collaborative Computerised Symptom Assessment (EPCRC). Their use within a clinical study might be considered if change in pain was considered to be the primary outcome measure (Melzack, 1975; Daut *et al.*, 1983; Melzack, 1987; Kaasa *et al.*, 2008).

Two scoring systems have been published specifically to monitor the extent and severity of oral lichen planus. The aim of these instruments was to allow objective monitoring of the disease over time and provide an outcome measure by which to assess the efficacy of treatments in clinical trials. The Escudier Index (Table 3) records the site of the lesion, its severity and also its activity (Escudier *et al.*, 2007). The Malhotra instrument (Table 4) groups anatomical areas together summarising the extent of the lesions by allocating more points to widespread disease (Malhotra *et al.*, 2008). The Escudier Index scores each clinical presentation of oral lichen planus (reticular, atrophic, desquamative, ulcerative and mixed) with a numerical value attributed to the severity of its presentation. The authors combined the clinical component with a subjective visual analogue scale (VAS) (0-100) to assess pain and assess the correlation between disease activity and pain. In a subgroup (n=23) they repeated the scores following 3 months of treatment with betamethasone 500μg mouthwash and conclusions suggest that the disease activity and pain scores were sensitive to clinical changes in the disease condition (Escudier *et al.*, 2007).

The clinical application of the indices described by Escudier et al. (2007) and Malhotra et al. (2008) were subsequently evaluated and correlated to pain and type of presentation (Lopez-Jornet and Camacho-Alonso, 2010b). They compared 100 biopsy proven patients with oral lichen planus and examined them according to the two scoring methods. The indices correlated with pain but the Escudier Index was more accurately able to differentiate between different forms of the disease (reticular vs mixed presentations).

Site	Site score (a)	Severity Score (b)	Activity Score (a x b)
Outer lips	(0 or 1)	0-3	0-3
Inner lips	(0 or 1)	0-3	0-3
Left buccal mucosa	(0,1 or 2)	0-3	0-6
Right buccal mucosa	(0,1 or 2)	0-3	0-6
Gingiva upper right	(0 or 1)	0-3	0-3
Gingiva upper central	(0 or 1)	0-3	0-3
Gingiva upper left distal	(0 or 1)	0-3	0-3
Gingiva lower left distal	(0 or 1)	0-3	0-3
Gingiva lower central	(0 or 1)	0-3	0-3
Gingiva lower right distal	(0 or 1)	0-3	0-3
Dorsum of tongue	(0,1 or 2)	0-3	0-6
Right lateral tongue	(0 or 1)	0-3	0-3
Left lateral tongue	(0 or 1)	0-3	0-3
Floor of mouth	(0,1 or 2)	0-3	0-6
Hard Palate	(0,1 or 2)	0-3	0-6
Soft Palate	(0,1 or 2)	0-3	0-6
Oropharynx	(0,1 or 2)	0-3	0-6
Maximum score	**24**	**51**	**72**

Table 3. Shows a scoring system for mucosal disease severity with special reference to oral lichen planus (Escudier *et al.*, 2007).
The mouth is divided into 17 sections and each attributed a score which reflects the extent and severity of the lesions
Site score (a) 0 = no detectable lesion, 1 = evidence of lichen planus, 2 = >50% of buccal mucosa, dorsum of tongue, floor of mouth, hard palate, soft palate, or oropharynx affected.
Severity score (b) 0 = keratosis only, 1 = keratosis with mild erythema (<3mm from gingival margin); 2 = marked erythema (e.g. full thickness of gingivae, extensive with atrophy or oedema on nonkeratinised mucosa); 3 = ulceration.
Activity is calculated by multiplying site score by severity score, (a x b).
The overall score is calculated by the site score + activity score + pain score (0-10) to give an overall maximum score of 106.

Site	Area involved	Points allotted
Right buccal mucosa	<50%	1
	>50%	2
Left buccal mucosa	<50%	1
	>50%	2
Tongue back surface	<50%	1
	>50%	2
Tongue front surface	<50%	1
	>50%	2
Upper lips	Uninvolved	0
	Involved	1
Lower lips	Uninvolved	0
	Involved	1
Gingiva	Uninvolved	0
	Involved	1
Palate	Uninvolved	0
	Involved	1
Maximum score		**12**

Table 4. Shows an objective clinical scoring system for assessing oral lichen planus (Malhotra et al. 2008).
It aims to classify and grade the severity of the disease based on the surface area affected. The patients are then graded based on the sum score of the points allotted; the severity is determined based on the grade where:
Grade 0 = 0 points; Grade 1 = 1-3 points; Grade 2 = 4-6 points; Grade 3 = 7-12 points

This is then interpreted by the clinician into three categories of oral lichen planus, mild, moderate and severe as follows:

Mild	Moderate	Severe
Asymptomatic grade 1 disease	Symptomatic grade 1 disease	Grade 3 disease
	Grade 2 disease	Erosive lesion of any grade

1.2 Social impact of disease

The 2012 systematic review into interventions for symptomatic oral lichen planus indicated that standardised pain and clinical assessments should feature in future studies and any adverse effects of treatment should be transparently reported (Lodi *et al.*, 2012). Pain scores may represent a common symptom but they do not capture other symptoms, which may be more pertinent to the patient. These might include an inability to eat, avoidance of particular foods that may exacerbate symptoms, chronic soreness, frequency of ulcers, aching, general discomfort or even changes in mood. Over recent years there has been a shift to understand, in a greater way, the effect that diseases impact on patients' lives. This should lead to treatment strategies that address the issues that are important to patients. Research has focussed on developing a series of surrogate instruments, often questionnaires that aim to assess the consequences of disease in this way. If these are to be used in a clinical setting or as outcome measures within clinical trials, an understanding is required of what they are designed to measure, the underpinning theory that fed into their development and in practical terms, how they should be administered.

Traditional theoretical models of health do not combine the more personal, human, psychosocial aspects of health and disease (George, 1978). Historically, the theoretical, biomedical model of health has regarded the human mind and body separately, in some ways almost mechanistically. This isolation of the body and the person ignores the patient's subjective experiences about health and illness with a greater emphasis on disease (Allen, 2003). The WHO defined health as "a complete state of physical, mental, and social well-being and not just the absence of disease" (World Health Organisation, 1948). It is essential that the effectiveness of health care interventions must still take the clinical findings into account but is inclusive of subjective experiences of health and well-being. The socio-environmental model of health has therefore challenged the traditional concept and accounts for subjective experiences of health, illness and demonstrates the need for a holistic approach to delivering healthcare (Labonte, 1993; Slade *et al.*, 1998; Allen, 2003).

1.2.1 Oral health

Oral health can be considered to be an integral part of general health. In clinical dentistry and oral health research, there is a tendency to consider the oral cavity (and its pathologies) as an isolated anatomical structure (Slade *et al.*, 1998). Defining oral health should account for the presence or absence of clinical pathology, as well as the social impact that health has on the individual. Various definitions of oral health exist that account the presence and absence of oral disease and include an acknowledgement of its social impact. Dolan defined oral health as "a comfortable and functional dentition, which allows individuals to continue in their desired social role" (Dolan, 1993). The UK Department of Health defines oral health as "a standard of health of the oral and related tissues which enables an individual to eat, speak and socialise without active disease or embarrassment and which contributes to general wellbeing" (Health, 2005a). Locker (1997) proposed that the focus should be much more at the individual level and in particular, the way in which oral conditions and diseases threaten health, well-being and quality of life (Locker, 1988; Locker, 1997). The conceptual model of oral health attempts to demonstrate the existence of relationships between oral health and impairment, function, discomfort and disability (Figure 6) (Locker, 1988; Locker, 1997). These definitions acknowledge the importance of being able to maintain social roles in comfort and function (Slade *et al.*, 1998).

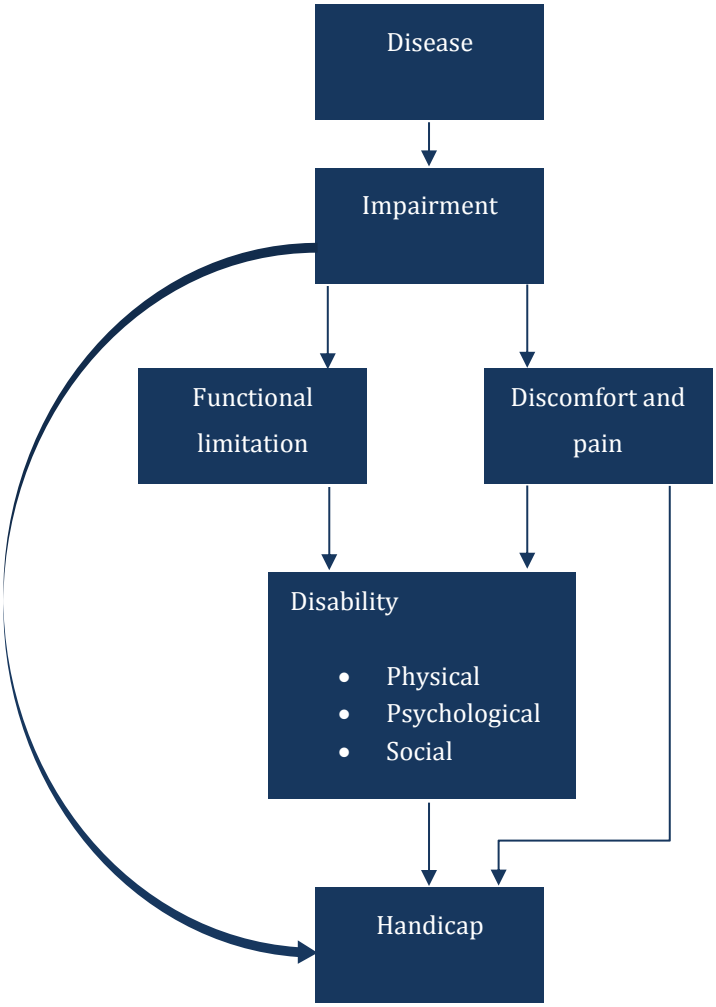


Figure 6. Locker's theoretical model of oral health.
This theoretical model, adapted from the original, forms the basis for a number of instruments that attempt to measure the social impact of oral conditions on well-being and quality of life.
This model comprises seven dimensions: functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability (shown collectively as disability) and handicap (Locker, 1988; Locker, 1997).

1.2.2 Quality of life

The terms health related quality of life (HRQOL) and quality of life (QOL) are often used interchangeably to define the social impact of health within medical contexts (Gill and Feinstein, 1994; Slade *et al.*, 1998; Carr *et al.*, 2001). Definitions vary from those with a holistic emphasis on the social, emotional and physical well-being of patients after treatment to others that describe the impact of a person's health on his or her ability to lead a fulfilling life (Greer, 1984; Bullinger *et al.*, 1993; Carr *et al.*, 2001). Despite these discrepancies there is a consensus that measures addressing quality of life should be patient-centred and reflect patients' perspectives (Leplege and Hunt, 1997; Locker and Allen, 2007). Some of these measures have been criticised for not addressing the concerns of patients and more accurately reflecting the views of the authors of the specific instruments (Leplege and Hunt, 1997).

Although diseases may impact on quality of life, this may not be true in every case, with healthcare professionals often assuming that poor health relates directly to poor quality of life (Allen, 2003). Measuring health related quality of life within interventional studies requires the use of validated, reliable instruments. Their use as an outcome measure within clinical trials has been recommended in conjunction with other clinical variables (Allison *et al.*, 1997; Sischo and Broder, 2011). Within medicine and dentistry, a multitude of different measures and instruments exist that aim to evaluate and measure this concept (Table 5). These are often self- or interviewer-administered questionnaires that have been developed and validated to measure differences in quality of life between patients at a particular point in time (discriminative instruments) or measure longitudinal changes within patients (evaluative instruments) (Guyatt *et al.*, 1993).

1.2.3 Development of quality of life outcome measures

A multitude of outcome measures have been developed to measure health related quality of life and oral health related quality of life (Table 5). The methods by which these measures have been developed originate from theoretical models and concepts or from the use of structured qualitative interviews to develop a series of statements or questionnaires.

Two types of patient-based outcome measures have been developed in an attempt to measure quality of life, a generic instrument that provides a summary of health

related quality of life and specific instruments that are focussed on problems associated with single disease states, patient groups or areas of function (Guyatt *et al.*, 1993). Generic health related quality of life instruments are designed to be relevant to anyone and, therefore, can be applied to a range of interventions and study populations. Disease targeted measures have been developed to identify features of heath related quality of life that are relevant to people with the condition, or disease of interest (Guyatt *et al.*, 1986; Vickrey *et al.*, 1992; Hays *et al.*, 1994; Wu *et al.*, 1997; Mangione *et al.*, 1998). These measures are potentially more sensitive than generic measures to smaller changes over time because they are selected to be particularly relevant to a given condition (Hays, 2005a). Ideally, the questionnaires should be developed following in-depth interviews of a sample of the population who will ultimately be asked to complete the final questionnaire or tool. These in-depth interviews should reveal a variety of experiences that are important to the respondents which can then be assimilated into statements or questions (Guyatt *et al.*, 1986). They must, however, be viewed in the context of overall health; in fact some advocate using generic and disease-targeted measures in combination to adopt the strengths of both approaches (Guyatt *et al.*, 1986; Guyatt *et al.*, 1993). Significant problems exist with the ever-increasing number of quality of life outcome measures (generic and disease targeted) and making comparisons between these different instruments as outcome measures even when examining the same disease or condition.

One of the earliest attempts at developing a multidimensional measure of health status was the Sickness Impact Profile (SIP). Its intended use was to measure change in the functional abilities of patients (Gilson *et al.*, 1975; Bergner *et al.*, 1981). The tool came under criticism for being too long (136 items) in comparison with the more widely accepted short forms (SF) comprising 12 (SF-12), 24 (Roland Scale) and 36 (SF-36) questions which were much easier to administer (Roland and Morris, 1983; Ware and Sherbourne, 1992; Slade *et al.*, 1998). Generic measures such as this have been used widely to compare the relative burden of disease between groups of patients.

Instrument	Author	Dimensions
Social Impacts of Dental Disease	Cushing *et al.*, 1986	Eating restrictions, communication restrictions, pain, discomfort and aesthetic dissatisfaction.
General (Geriatric) Oral Health Assessment Index (GOHAI)	Atchison and Dolan, 1990	Physical function (including eating, speech and swallowing), Psychosocial (including worry or concern about oral health, dissatisfaction with appearance, self-consciousness about oral health and avoidance of social contacts because of oral problems), Pain and discomfort (including the use of medication to relieve pain or discomfort from the mouth).
Dental Impact Profile (DIP)	Strauss and Hunt, 1993	Eating (eating, chewing, biting, enjoyment of eating, food choice, tasting), Health/Well-being (feeling comfortable, enjoyment of life, general happiness, general health, appetite, weight, living a long life), Social relations (facial appearance to self and others, smiling and laughing, moods, speech, breath, confidence around others attendance at activities, success at work), Romance (social life, romantic relationships, having sex appeal, kissing).
Oral Health Impact Profile (OHIP)	Slade and Spencer, 1994	Functional limitation (difficulty chewing), physical pain (sensitivity of teeth), psychological discomfort (self-consciousness), physical disability (changes to diet), social disability (avoiding social interaction), handicap (being unable to work productively).
Subjective Oral Health Status Indicators (SOHSI)	Locker and Miller, 1994	Ability to chew, ability to speak, oral and facial pain symptoms, other oral symptoms, eating impact scale, activities of daily living impact scale, worry/concern impact scale.
Oral Impacts on Daily Performance (OIDP)	Adulyanon *et al.*, 1996	Performance in eating, speaking, oral hygiene, sleeping, appearance, emotion.
Oral Health Related Quality of life (OHQOL)	Kressin *et al.*, 1996	Effect on daily activities such as work or hobbies, affected social activities with family friends or co-workers, avoids conversations with people because of appearance.
Dental Impact on Daily Living (DIDL)	Leao and Sheiham, 1996	Comfort (related to bleeding gums and food packing), Appearance (consisting of self-image, pain, performance (the ability to carry out daily activities and to interact with people, eating restriction (relating to difficulties in biting and chewing).
Oral Health Quality of life Inventory (OH-QoL)	Cornell *et al.*, 1997	Importance and satisfaction of oral health and functional status.
Rand Dental Questions	Dolan and Gooch, 1997	Pain, worry or concern, reduced social interactions.

OHRQoL for Dental Hygiene	Gadbury-Amyot *et al.*, 1999	Symptom status, functional status, health perceptions.
Orthognathic QoL Questionnaire	Cunningham *et al.*, 2000	Social aspects of deformity, facial aesthetics, function, awareness of facial deformity.
OHQoL-UK	McGrath and Bedi, 2001	Physical, social, psychological.
Child Oral Health Quality of life Questionnaire (COHQoL)	Jokovic *et al.*, 2002	Oral symptoms, functional limitations, emotional well-being, social well-being (peer interaction, schooling and leisure activities).
Child OIDP	Gherunpong *et al.*, 2004	Eating, speaking, cleaning mouth, doing activity, sleeping, emotion, smiling, emotion, study, social contact.
Liverpool Oral Rehabilitation Questionnaire	Pace-Balzan *et al.*, 2004	Oral Function, dentures/denture satisfaction.
Surgical Orthodontic Outcome Questionnaire (SOOQ)	Locker *et al.*, 2007	Function 1 (issues before surgery), function 2 (issues after surgery), dental aesthetics, facial aesthetics, emotional and social well-being.
Outcomes of Prosthodontic Treatment	Leles *et al.*, 2008	Benefits, risks, perceived consequences of treatment or no treatment.
Chronic Oral Mucosal Disease Questionnaire (COMDQ)	Ni Riordain *et al.*, 2011	Pain and functional limitation, medication and treatment, social and emotional, patient support.

Table 5. Shows the measures of oral health related quality of life, adapted from Locker and Allen (2007).
Many shortened versions of these questionnaires have also been published and the questionnaires translated into different languages (Locker and Allen, 2007).

1.3 Oral health related quality of life instruments

The dental profession and the general population have markedly different perceptions about what the outcomes of dental treatment should be (Kay and Blinkhorn, 1996). There may be consequences of oral disease that go far beyond the clinical presentation including alterations to self-esteem; social interaction; diet; education and job performance (Allen *et al.*, 1999; Sischo and Broder, 2011). The term 'oral health related quality of life' under the umbrella of patient-based outcomes, has replaced earlier terms like socio-dental indicators, oral health status, subjective oral health or social impacts of oral disease that were used to describe factors that drew on patients' perspectives about their oral health (Locker and Allen, 2007; Tsakos *et al.*, 2012). Research followed that then attempted to understand the interrelationships between traditional clinical findings, the presence or absence of disease and patient-centered, self-reported health (Gift and Atchison, 1995; Sischo and Broder, 2011).

The generic Sickness Impact Profile has been used to assess the social impact of oral and dental disease including in temporomandibular joint dysfunction, periodontitis, prosthodontics and those presenting for routine recall (Slade *et al.*, 1998). Whilst it was used with some success where the impact of the oral conditions was expected to be high, the lack of sensitivity to changes in oral functional status have led to it (and other generic methods) being infrequently used to evaluate the social impact of oral conditions (Reisine, 1988; Reisine *et al.*, 1989; Reisine and Weber, 1989; Slade, 1998).

Following the initial proposal that social indicators should be developed and used within dentistry, Reisine and co-workers were among the first to use a multitude of validated scales to determine the impact of dental conditions on quality of life (Cohen and Jago, 1976; Reisine, 1981; Reisine *et al.*, 1989). There are now a significant number of instruments that exist and are dedicated to assessing oral health related quality of life (Table 5) (Locker and Allen, 2007).

The aim of these measures, usually questionnaires, is to assess the consequences of impaired oral health from the patient's perspective, attempting to measure the impact of oral health on quality of life (Birch and Ismail, 2002; Ng and Leung, 2006). Many of these have been used in population surveys or clinical trials to

measure the social impact of oral disorders (Locker and Allen, 2007). It is important to understand what each of the evaluative instruments is trying to measure and to distinguish between a measure of oral health status and a measure of oral health related quality of life. In a trial or study the evaluative tool chosen must be relevant to the population being examined. Ideally it should be developed from responses of a similar cohort of people to those who will ultimately complete the questionnaires or surveys (Guyatt *et al.*, 1986; Locker and Allen, 2007). Evaluating the large number of health related quality of life and oral health related quality of life measures that have been developed requires a systematic approach to assess their applicability, reliability and validity for use in research (Gill and Feinstein, 1994; Guyatt and Cook, 1994; Locker and Allen, 2007).

If any of the existing patient-based outcome measures are to be utilised, they should attempt to assess the broader meaning and context of the social effects of oral health, particularly in relation to overall health (Locker and Allen, 2007; Tsakos *et al.*, 2012). These authors also suggest that this may be partly achieved through the concurrent use of global ratings which can accommodate individual meaning and significance of disease-related events (Locker and Allen, 2007). Oral health related quality of life measures have been utilised in clinical research studies as primary and secondary outcome measures, in epidemiological studies and health services research to identify trends in oral health (Sischo and Broder, 2011; Tsakos *et al.*, 2012). It is the outcomes of population-based studies that are most likely to influence healthcare promotion and provision; identifying specific groups which are likely to have low oral health related quality of life and may benefit from tailored oral health programmes (Sischo and Broder, 2011).

Clinical studies using patient-based outcomes have evaluated the effectiveness of interventions on quality of life; despite their existence there are a limited number in oral medicine and fewer still related to oral lichen planus (Hegarty *et al.*, 2002a; Hegarty *et al.*, 2002b; McGrath *et al.*, 2003b; Gorouhi *et al.*, 2007; Heffernan *et al.*, 2007; Lopez-Jornet *et al.*, 2009; Ni Riordain and McCreary, 2010). Generic, oral specific and condition specific quality of life outcome measures have been used in an attempt to calculate the degree that conditions presenting to oral medicine clinics which include oral lichen planus, Behçet's, recurrent aphthous ulceration, pemphigus vulgaris and burning mouth syndrome, impact on quality of life

(Hegarty *et al.*, 2002b; McGrath *et al.*, 2003b; Paradisi *et al.*, 2009; Tabolli *et al.*, 2009; Ni Riordain and McCreary, 2010; Ni Riordain and McCreary, 2012). The generic SF-36, EuroQol, and Hospital Anxiety and Depression (HAD) scales have been used separately and in conjunction with other generic health related quality of life indices in studies of oral mucosal disease (Paradisi *et al.*, 2009; Tabolli *et al.*, 2009). More frequently, they have been used in conjunction with the oral health related quality of life questionnaires such as the full and shortened versions of the oral health impact profile (Lopez-Jornet *et al.*, 2009; Salazar-Sanchez *et al.*, 2010; Liu *et al.*, 2012). The most frequently utilised measures are the Oral Health Impact Profile (OHIP-49), its shortened version (OHIP-14) and the OHQoL-UK. More recently a condition specific quality of life questionnaire has been developed for chronic oral mucosal diseases (COMDQ) (Hegarty *et al.*, 2002a; McGrath *et al.*, 2003a; McGrath *et al.*, 2003b; Lopez-Jornet *et al.*, 2009; Salazar-Sanchez *et al.*, 2010; Ni Riordain *et al.*, 2011; Liu *et al.*, 2012; Ni Riordain and McCreary, 2012).

In oral lichen planus research, a robust approach to identifying and using appropriate outcome measures has not been seen, some have used multiple measures of oral health related quality of life (OHQoL-UK and OHIP-14), multiple methods of assessing pain (McGill Pain Questionnaire and VAS scales) alongside clinical indices (Hegarty *et al.*, 2002a). Few studies that use quality of life outcomes measures in oral medicine research have attempted to contextualise the findings with change in size or severity of oral lesions (Hegarty *et al.*, 2002a). Those that have, more appropriately, used a single oral health related quality of life measure in combination with a global scale and visual analogue scales to provide more context and meaning of the quality of life outcome measure (McGrath and Bedi, 2003).

If a new quality of life instrument is to be developed or one chosen from an existing array of instruments (Table 5) careful consideration should be given to examine the characteristics of the individual instruments and whether they are the most applicable instruments to use. The psychometric properties of a measure should reveal reliability, validity and the instrument should be relatively straightforward to use (Guyatt *et al.*, 1987). The authors of an instrument should be able to demonstrate that it measures the same outcome consistently; test-retest reliability is one such evaluation and should be investigated during the

development of a questionnaire (Kirshner and Guyatt, 1985). Reliability is further complicated by changes that may have occurred between administrations of the questionnaires over time, therefore reliability is key in longitudinal studies (Carr *et al.*, 2001; Hays, 2005b). Validity is the degree to which the instrument assesses what it is intended to measure. This differs to the concept of reliability, whilst a measure may always provide identical scores for the same patient, it may be consistently measuring the wrong outcome. The evidence for an instrument's validity is continuous and a patient-based outcome measure must, therefore, be shown to be both valid and reliable. When identifying which measure to use, the validity of that measure for a particular group or population must be evaluated (Hays, 2005b). Although a measure may be reliable and valid, it may not have the ability to capture small but meaningful changes over time. Responsiveness, therefore, ultimately reflects any underlying change (Guyatt *et al.*, 1987; Hadorn and Hays, 1991). If validity, reliability and responsiveness have been demonstrated for a measure, comparisons can be made between clinical status and quality of life (Chambers *et al.*, 1987).

In quality of life research, investigators need to have confidence that a difference, where one exists, will be detected (Guyatt *et al.*, 1993). Responsiveness is related to the magnitude of the difference in score in patients who have improved or deteriorated (the signal) and the extent to which patients who have not changed provide the same scores (the noise). Responsiveness to change is most frequently assessed using effect size, standard response mean and/or a responsiveness statistic. With respect to the magnitude of change in response to the intervention: an effect size of 0.2 represents a small change, 0.5 a medium change and 0.8 a large change (Cohen, 1992). Whilst this may be statistically significant, interpreting the clinical significance can be difficult. Within quality of life research, the term minimally important clinical difference has been used to contextualise scores produced by quality of life measures (Jaeschke *et al.*, 1989; Juniper *et al.*, 1994; Locker *et al.*, 2004; Allen *et al.*, 2009; Tsakos *et al.*, 2012). This also allows an *a priori* calculation of the sample size needed to detect a certain effect size for a particular measure (Hays, 2005b). Assessments of minimally important difference are particularly important where patient-based outcomes are the sole outcome measure and there are no other (clinical) anchors. Of the more commonly used measures of oral health related quality of life, the minimally important difference

for the OHIP-14 was estimated to be 5 points, the OHIP-20 between 7-10 points, the OHIP-49, 6 points and the OIDP, 5 points (Locker *et al.*, 2004; Allen *et al.*, 2009; John *et al.*, 2009).

1.3.1 The Oral Health Impact Profile

The Oral Heath Impact Profile (OHIP) was developed with the aim of providing a comprehensive measure of self-reported dysfunction, discomfort and disability attributed to oral conditions (Slade and Spencer, 1994; Slade, 1997). It has been described as the most comprehensive measure of the impact of oral conditions on quality of life (Allen *et al.*, 1999). The OHIP is the most widely used measures of oral health related quality of life that has been shown to be valid and reliable. It has also been translated into a number of languages and used successfully as primary and secondary outcome measures in clinical trials (Allen and Locker, 2002; Hegarty *et al.*, 2002a; John *et al.*, 2002; Allen and McMillan, 2003; Ekanayake and Perera, 2003; Ikebe *et al.*, 2004; John *et al.*, 2004b; Kushnir *et al.*, 2004; Larsson *et al.*, 2004; Heydecke *et al.*, 2005; Wolfart *et al.*, 2005; Allen *et al.*, 2006; Pires *et al.*, 2006; Al-Jundi *et al.*, 2007; Barer *et al.*, 2007; Heffernan *et al.*, 2007; Ozcelik *et al.*, 2007; Saub *et al.*, 2007; Hassel *et al.*, 2008; Rener-Sitar *et al.*, 2008; Wostmann *et al.*, 2008; Linsen *et al.*, 2009). Originally developed for measuring the impact of oral disorders in epidemiological research, OHIP was based on the seven conceptual dimensions of oral health proposed by Locker (Locker, 1988):

- Functional limitation (for example difficulty chewing);
- Physical pain (for example sensitivity of teeth);
- Psychological discomfort (for example self-consciousness);
- Physical disability (for example reduced ability to concentrate);
- Social Disability (for example avoiding social interaction);
- Handicap (for example being unable to work properly).

The OHIP consists of a 49-point questionnaire (Appendix 1). Initially statements rather than questions were produced following structured interviews using open-ended questions. The subjects were drawn from a convenience sample of 64 adult dental patients in Adelaide, Australia. 535 distinct statements were initially scrutinised resulting in 46 individual statements. These were then classified into the seven conceptual dimensions (Slade and Spencer, 1994). Three further statements were added to the handicap dimension before all 49 statements were rephrased as questions (Hunt *et al.*, 1985). The method by which the items were

selected reflected their fit with Locker's conceptual framework. As such the selection was expert-centred rather than accounting for the relative importance to the patients from which they were initially developed. The questionnaire was not designed to measure any positive aspects of health and relates to general oral conditions rather than specific oral disorders or syndromes. OHIP attempts to measure the burden of illness and provides information on the frequency rather than severity of the impact of oral health.

Respondents to the original questionnaire were provided with a 5-point Likert scale to rate the frequency that each problem was experienced over a reference period of twelve months. Responses on the 5-point Likert scale were coded 0 (never or not applicable), 1 (hardly ever), 2 (occasionally), 3 (fairly often), 4 (very often). The qualitative nature of the responses can then be quantified and the data interpreted and analysed. Frequency can also be ascertained by calculating the sum of the impacts (Slade *et al.*, 1998). In the original study, each question was also weighted according to Thurlstone's method of paired comparisons (Thurlstone, 1927). Subsequent studies have suggested that simple scoring methods were as good as more sophisticated ones despite item weights improving the performance of OHIP (Allen and Locker, 1997). The reliability of OHIP to capture oral health related quality of life has been assessed on a number of occasions and in subsequent translations of the measure (John *et al.*, 2002; Wong *et al.*, 2002; Larsson *et al.*, 2004; Szentpetery *et al.*, 2006; Al-Jundi *et al.*, 2007; Bae *et al.*, 2007; Saub *et al.*, 2007; Souza *et al.*, 2007; Yamazaki *et al.*, 2007).

Shortened Versions of the OHIP

There are a number of shortened versions of the Oral Health Impact Profile (OHIP) for use in different settings and for different patient populations. The original short version (Slade, 1997) used regression analysis to derive a 14-question version (OHIP-14) from the original OHIP instrument. Locker and Allen (2002) derived their shortened version of OHIP using the item impact method, which only had two items in common with the version published by Slade (1997). The regression short form is considered to be better when the aim is to discriminate while the impact short form is preferable when describing the oral health-related quality of life of populations or when working to detect a change (Slade, 1997; Locker and Allen, 2002).

1.3.2 Practicalities of administration

Questionnaire based tools can be either self-administered or managed by a trained interviewer; the method of administration has the potential to influence the results and completion rates of the questionnaire. Interviewer administration is resource intensive but ensures compliance, decreases errors and decreases missing items (Guyatt *et al.*, 1993). Self-administration is much less expensive, but increases the number of missing responses and missing subjects (Guyatt *et al.*, 1993). In a study by Robinson and co-workers (2001) the completion rate of OHIP-14 and Oral Impacts on Daily Performance (OIDP) questionnaires were similar in interview format although in questionnaire format, usable data were provided on 92.9% of the OHIP-14 responses and only in 86.5% of OIDP responses. They concluded that the method of administration did not affect the psychometric properties of the OHIP-14 and OIDP questionnaires (Robinson *et al.*, 2001). In the medical field both self- and interviewer-administered questionnaires yielded similar results demonstrating the ability to discriminate between individuals. Self-administered questionnaires, however, were associated with greater health related quality of life impairment and may be more appropriate to evaluate the true impact of disease (Cook *et al.*, 1993).

The majority of the measures of oral health related quality of life have good psychometric properties, in addition they have been shown to be reliable, valid and responsive to clinical change (Locker and Allen, 2002). Although interviewer-administered questionnaires may increase response rates, Locker and Allen proposed that longer scales are more likely to be subject to item non-response, giving rise to problems of how to manage missing data. The use of these instruments in some clinical settings and population-based surveys may be limited by questionnaire length and the complexities involved in completing the instruments (Locker and Allen, 2002). The distillation of measurements to form reliable shortened questionnaires has been a goal for clinical investigators to improve the efficiency of patient and clinical time (Ware and Sherbourne, 1992; Guyatt *et al.*, 1993; Coste *et al.*, 1997; Guillemin *et al.*, 1997; Allen and Locker, 2002). One approach to this problem has been to develop a long instrument, test it and use its performance to choose key questions to include in a shorter index. This approach has been used to create shorter questionnaires based on the lengthy instruments from the Medical Outcomes Survey and to develop shortened versions

of the Oral Health Impact Profile (OHIP) (Stewart *et al.*, 1988; Slade, 1997; Allen and Locker, 2002; Locker and Allen, 2002; Broder *et al.*, 2007; Durham *et al.*, 2011).

Questionnaires evaluating subjective oral health status use a reference period of time in which respondents are asked to recall their experiences of oral health (Sutinen *et al.*, 2007). The length of the reference period may have marked influence on the responses obtained. The Oral Heath Impact Profile has a standardised reference period of 12 months, however for some studies this reference period is not practical. Investigations into the reference period of this measure have shown that for use in population surveys the shortening of this reference period did not seem to influence the responses (Sutinen *et al.*, 2007). Other questionnaires have successfully used different reference periods: for example 1 or 6 months instead of 12 months (John *et al.*, 2004a; Allen *et al.*, 2006).

Item Weights

The scoring methods used by some quality of life instruments simply involve summing up the item scores with each given equal weighting (Atchison and Dolan, 1990). It has been proposed that some items within questionnaires may be more important to the underlying concept of oral health related quality of life than others (McGrath and Bedi, 1999; McGrath and Bedi, 2004). As such, other instruments use a 'weighting' scoring system that incorporates an assessment of the importance or severity of oral health on quality of life (Leao and Sheiham, 1995; Slade, 1998; Sheiham *et al.*, 2001; McGrath and Bedi, 2004). Different questionnaires have used various methods to weight questionnaires; some weight the measure at an individual level, others at the domain level (McGrath and Bedi, 2004). Some have also argued that weighting questions in this way is not necessary and has little or no advantage over using un-weighted, summing of the response scores (Trauer and Mackinnon, 2001; McGrath and Bedi, 2004).

The Oral Health Impact Profile and the OHQoL-UK(W)© were both found to have similar psychometric properties when used in weighted or unweighted forms and their use yielded no additional benefit (Allen and Locker, 1997; McGrath and Bedi, 2004). McGrath and Bedi (2004) did however discuss the need for further work on item weighting when specific clinical conditions are assessed or in longitudinal studies (McGrath and Bedi, 2004).

1.3.3 Global self-rating

The concept of self-rated health is not assessed in all generic or disease-specific instruments. Asking individuals to rate their health on a scale ranging from excellent to poor has become standard practice in population-based health surveys and health evaluations (Locker *et al.*, 2005). It has been argued that all health related quality of life instruments should include at least one simple global question about overall health and/or overall quality of life (Bjorner *et al.*, 2004). It has been proposed that in this way, respondents can weight together different aspects of their health status emphasising those aspects of health that they consider to be most relevant (Gill and Feinstein, 1994). Single item ratings can be as useful as more complex, multi-item scales and indices in health status assessment (Ware *et al.*, 1993; Locker *et al.*, 2005). One study looked at the relationship between global self-rating, oral health related quality of life and self-reported treatment need. They identified that the global rating varied over time and that the changes were consistent with those measured by both these tools (Dolan *et al.*, 1998). Others have gone one stage further to suggest that if only one question was allowed to measure health status, the rating of health as excellent, very good, good, poor or fair would be a good candidate (Bjorner *et al.*, 2004).

Global self-ratings are also used to test the construct validity of patient-based measures of health related quality of life (Locker *et al.*, 2005). There is also evidence that these ratings are powerful predictors of functional decline and survival and are predictors of the use of health care services (Evashwick *et al.*, 1984; Idler and Benyamini, 1997; Shadbolt *et al.*, 2002; Locker *et al.*, 2005). Others have criticised the use of these measures as being less precise than more extensive multi-item instruments, if used as the only outcome measure, with the lack of precision and sensitivity being a potential disadvantage in small trials (McHorney *et al.*, 1992).

1.4 Biomarkers of inflammation

Periodontal diseases arise from inflammation of the supporting structures of the teeth and can be subdivided into two basic categories, gingivitis and periodontitis. Gingivitis is more superficial inflammation confined to the gingival tissues and is reversible with effective, simple interventions such as tailored oral hygiene advice and professional prophylaxis (Armitage, 1999). Periodontitis is irreversible and affects the deeper structures leading to progressive breakdown of the periodontal ligament and alveolar bone (Savage *et al.*, 2009). Bacterial infection is the primary causative agent of both gingivitis and periodontitis with certain species being associated with more destructive disease. The presence of gingivitis has been demonstrated in animal studies to be a precursor to periodontitis, however progression in human subjects is not always linear (Heijl *et al.*, 1976; Socransky *et al.*, 1984; Löe *et al.*, 1986). There are differences between individuals in their susceptibility to periodontal diseases, which may be due to variations in environmental exposures or genetic factors or a combination of both (Page and Kornman, 1997).

The most widely utilised methods for diagnosing diseases affecting the periodontium and assessing outcomes in trials are clinical signs such as tissue colour and contour, presence or absence of bleeding on probing, gingival recession, probing pocket depths and attachment levels, suppuration, tooth mobility and radiographic assessment of bone loss (Lindhe *et al.*, 1986; Buduneli and Kinane, 2011). Some of these are subjective but those that can be quantified, such as pocket probing depths, are subject to variations in reproducibility. These all provide retrospective assessments eliciting evidence of past disease progress; they give very little information regarding susceptibility of future disease.

Biological indicators or biomarkers are biochemical, genetic or molecular substances that are indicative of a clinical disease or pathology. They can provide a more objective assessment by which to diagnose inflammatory disease and predict or assess future risk (Buduneli and Kinane, 2011). Periodontal diseases have complex aetiology and one single marker is unlikely to address all the issues relating to diagnosis and prognosis; the use of a combination of tests may provide a better understanding of these complex interactions in order to implement timely preventive strategies or initiate targeted therapy (Buduneli and Kinane, 2011).

Molecules in gingival crevicular fluid (GCF) and saliva as well as in blood, serum or plasma have been examined to determine a sensitive and specific marker for periodontal disease susceptibility (Buduneli and Kinane, 2011).

The site-specific nature of periodontal disease led to many research studies focussing on collecting biomarkers from sites that were easily accessible such as the gingival sulcus by collecting GCF. GCF has become the medium of choice through which inflammatory biomarkers are collected and analysed. Biomarkers have been used in periodontal research as objective outcome measures in clinical trials (Heasman *et al.*, 1993; Gamonal *et al.*, 2000; Offenbacher *et al.*, 2007; Offenbacher *et al.*, 2010). GCF is a serum transudate that is described as being enriched with microbial and host products that arise from the dynamics of the host-biofilm interaction and is therefore the ideal medium to look at gingival inflammation (Offenbacher *et al.*, 2010). It originates from the gingival plexus of blood vessels in the gingival connective tissue close to the epithelial lining of the gingival sulcus and provides a good representation of tissue and serum concentrations of inflammatory mediators (Armitage, 2004; Buduneli and Kinane, 2011). There have been over 90 components of GCF that have been evaluated as possible biomarkers for diagnosis of periodontal disease; some are host-derived enzymes and their inhibitors, inflammatory mediators and by-products of tissue destruction (Loos and Tjoa, 2005; Lamster and Ahlo, 2007).

Whole saliva is easily collected and contains exocrine gland secretions, GCF, dietary and oral plaque. It represents a pooled sample with contributions from all periodontal sites and provides an overall assessment not a site specific analysis (Silverman *et al.*, 1985). Several mediators of chronic inflammation and tissue destruction have been detected in whole saliva however in elderly patients collecting saliva samples may prove challenging due to hypo-salivation (Kaufman and Lamster, 2000; Buduneli and Kinane, 2011).

Pro-inflammatory cytokines also play a significant role in the pathogenesis of both hard and soft tissue destruction, interleukin-1α (IL-1α), interleukin 1-β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-18 (IL-18) and tissue necrosis factor-α (TNF-α) are all expressed in human gingiva. Increased levels of several of these mediators of inflammation, (IL-1α, IL-1β, IL-6, IL-8, IL-18) have been reported in sites exhibiting signs of gingivitis and periodontitis (Offenbacher *et al.*,

2007; Toker *et al.*, 2008; Pradeep *et al.*, 2009; Teles *et al.*, 2009; Fitzsimmons *et al.*, 2010; Offenbacher *et al.*, 2010). Matrix metalloproteinases (MMPs) are considered to be modifiers of host response and in pathological tissue destruction, in particular degradation of extracellular matrix and basement membrane (Buduneli and Kinane, 2011). They are divided into five groups: collagenases (MMP-1, MMP-8, MMP-13); gelatinases (MMP-2, MMP-9); stromelysins (MMP-3, MMP-10, MMP-11); and membrane type (MMP-14, MMP-15, MMP-16, MMP-17). It is the interrelationships and interactions between the cell-cell and cell-matrix involving enzyme production, activators, inhibitors, cytokines and growth factors that regulates connective tissue remodelling and connective tissue matrix destruction (Reynolds and Meikle, 1997; Buduneli and Kinane, 2011). The tissue inhibitors of MMPs (TIMPs) control the MMPs that are produced by fibroblasts, macrophages, neutrophils and epithelial cells (Buduneli *et al.*, 2007). Experimental gingivitis *in vivo* studies have demonstrated the presence of markers of neutrophil activation and recruitment, such as interleukin-8 (IL-8) and neutrophil degranulation such as β-glucuronidase, elastase and other matrix-metalloproteinases (MMPs) (Heasman *et al.*, 1993; Lamster *et al.*, 1994; Soder *et al.*, 2002; Offenbacher *et al.*, 2010). Clinical diagnoses have correlated with a change in MMP levels in GCF, saliva and blood samples (serum or plasma) (Alpagot *et al.*, 2001; Kinane *et al.*, 2003; Alfant *et al.*, 2008; Passoja *et al.*, 2008). Changes in biomarkers as outcome measures within longitudinal studies and prospective clinical trials have also been assessed (Pozo *et al.*, 2005; Chapple *et al.*, 2007; Golub *et al.*, 2008; Toker *et al.*, 2008; Gapski *et al.*, 2009; Offenbacher *et al.*, 2010).

The use of protein-based suspension bead multiplex immunoassays allows the simultaneous analysis of multiple inflammatory mediators. In an experimental gingivitis study, 16 separate inflammatory mediators were analysed that were associated with gingivitis (Offenbacher *et al.*, 2007).

As well as gingival crevicular fluid, the accessibility of the periodontal pocket also allows for samples of supra- and sub-gingival plaque to be collected and analysed for the presence or absence of certain bacterial species associated with healthy periodontium, gingivitis and periodontitis. There are more than 700 bacterial species that have been detected in the oral environment. Some are considered important for maintaining oral health whereas others are implicated in the

pathogenesis of oral diseases such as dental caries and periodontitis (Socransky *et al.*, 2002; Hojo *et al.*, 2009). Some bacteria, such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola, Tanerella forsythia* are specifically associated with more destructive periodontal diseases (Socransky *et al.*, 1998). They are supplied by the wide availability of nutrients from saliva, GCF, food debris as well as the metabolites produced by other organisms. The gingival sulcus is an ideal environment for bacteria to thrive (Hojo *et al.*, 2009). Bacteria do not live in isolation but exist in complex biofilms, and since some biofilms exist in health it has been proposed that certain commensal bacteria may have a protective role in excluding pathogenic bacteria although, the role of individual species within the microbiological ecology and their interactions with the host are not particularly well understood (Kumar *et al.*, 2006; Hojo *et al.*, 2009). Bacteria within a dental biofilm will interact with one another co-operatively and competitively with other species, the metabolites that they produce will also impact on the host and the type of response that is produced (Hojo *et al.*, 2009). Bacterial culturing is considered to be the classical diagnostic method to study bacterial species in dental plaque; it is also the standard against which new technologies should be measured against (Lau *et al.*, 2004; D'Ercole *et al.*, 2008).

Cultivation techniques and subsequently nucleic acid amplification methods such as PCR have been used to target bacteria obtained from small sample volumes (MacFarlane *et al.*, 1988; Listgarten *et al.*, 1991; Machtei *et al.*, 1997; Tanner *et al.*, 1998; Kumar *et al.*, 2006). Some bacteria, in particular anaerobic bacteria, are sometimes difficult to culture and analyse. Advances in technology, and in particular the cloning and sequencing of bacterial 16s rRNA genes, has allowed many previously uncultured bacteria to be identified (Kumar *et al.*, 2006). Immunoassays target and identify specific bacteria by using monoclonal or polyclonal antibodies against specific bacterial antigens. Methods include the enzyme linked immunosorbent assay (ELISA), multiplex flow cytometry, and direct or indirect immunofluorescent microscopy.

1.4.1 Potential biomarkers in oral lichen planus

Pilot studies have looked at levels of biomarkers in patients with oral lichen planus (Thornhill, 2001; Khan *et al.*, 2003; Rhodus *et al.*, 2005; Rhodus *et al.*, 2007; Zhang

et al., 2008). These studies have used whole unstimulated saliva, a mixture of saliva and isotonic saline rinse, lesion tissue transudate, blood serum, biopsy tissue samples to measure different cytokines and MMPs (Khan *et al.*, 2003; Rhodus *et al.*, 2005; Rhodus *et al.*, 2007; Zhang *et al.*, 2008). Many of the cytokines that have been investigated within the periodontal disease model are not specific and are part of the innate immune response; it is likely that they will have a role in lichen planus. Cytokine release is fundamental to functioning T-cells; in particular IL-1α and IL-1β are involved in T-cell activation and will be produced in response to a variety of stimuli. IL-2 is considered to be a potent T-cell growth factor and involved in long-term proliferation of activated t-cells and it is this pathway that is inhibited in immunosuppressive treatments (Simark-Mattsson *et al.*, 1999; Zhao *et al.*, 2002). T-Cells, neutrophils, basophils and eosinophils are chemically attracted to areas by gradients set up by the CXC chemokines (e.g. IL-8) and the CC cytokines (e.g. MIP-1α, MIP-1β, RANTES) (Baggiolini *et al.*, 1997; Zlotnik and Yoshie, 2000; Little *et al.*, 2003).

1.5 Health Economics

Within healthcare there are finite resources, so careful consideration should therefore be given to the most appropriate treatment options to provide (Heasman *et al.*, 2011). Within dentistry, cost-effectiveness is recognised as an important aspect of evaluating dental treatment and interventions (Antczak-Bouckoms and Weinstein, 1987; Braegger, 2005; Pennington *et al.*, 2009a; Pennington *et al.*, 2009b). Consideration should be given to which treatments are: effective (capable of achieving objective); available (does it reach those who need it?); efficient (is it the best use of time and resource?); and efficacious (has the capacity for beneficial change) (Drummond *et al.*, 2005). The focus of an economic evaluation is concerned with comparing costs and benefits, but very few of these evaluations have been carried out in dentistry, and even fewer in oral medicine (van der Meij *et al.*, 2002; Pennington *et al.*, 2009a; Pennington *et al.*, 2009b). A treatment that is cost-effective is one for which the benefits of that treatment exceed the costs (Pennington *et al.*, 2009a; Pennington *et al.*, 2009b). In dentistry, the current literature has investigated: caries prevention; third molar removal; root canal treatment vs dental implants; local and systemic antibiotics for management of periodontal disease; and supportive periodontal care. The majority of dentistry within the UK is provided through the NHS and patients will normally pay towards the cost of their treatment. When examining health economics, the costs should also be evaluated from the patients' perspectives, which may include loss of earnings, time travelled to and from the appointment and out of pocket expenses for travel costs (Gaunt *et al.*, 2008). There are various types of economic evaluation: the cost-benefit analysis, cost-effectiveness analysis and cost-utility analysis which differ in the way they value benefits (Pennington *et al.*, 2009a). A cost-benefit analysis attaches a monetary value to the benefit, in contrast to the cost-utility analysis which uses a quality of life measure such as the generic quality adjusted life year (QALY) or the oral health impact profile. A cost-effectiveness analysis compares outcomes on an appropriate quantitative scale (Gold *et al.*, 1996). A common misconception is that the benefits of treatment are simply the costs avoided by that treatment, whilst the costs avoided should be included, as a negative cost (Pennington *et al.*, 2009a). There is a need to assign costs correctly when carrying out an economic analysis and to determine both the incremental cost and incremental benefit of treatment compared to the alternatives. This is

calculated by [all costs arising from and following treatment] – [all costs arising from and following the alternative intervention] (Pennington *et al.*, 2009a). If an economic analysis is conducted as part of a clinical trial, all economic issues can be considered in the design of the trial and the ideal outcomes for the economic analysis can be planned and recorded (Gaunt *et al.*, 2008).

1.6 Translating research

General dental practitioners (GDPs) and other dental care professionals will normally tailor the advice that they provide to patients based on their own knowledge and expertise as well as adhering to clinical guidelines (Newman, 1996). For much of the 60 years of NHS dentistry, its main focus has been that of delivering treatment rather than focussing on prevention or quality. A recent review into NHS dentistry has suggested that future contracts must incentivise general practitioners into providing a high quality service and educating their patients in order to improve health (Steele, 2009).

The aim of preventive dentistry is to educate patients so that they can prevent many of the common oral diseases such as caries and periodontitis. As novel research is published and technological advancements in techniques and materials occur, dentists must be in a position to evaluate these innovations and make decisions whether or not to implement these findings and translate them into their own clinical practice.

Dentistry is almost entirely carried out within primary care, either through the NHS or in the private sector, with a limited number of more specialised services taking place in tertiary referral centres (Haines and Donald, 1998; McGlone *et al.*, 2001; Chapple *et al.*, 2003). The cost burden in NHS primary care dentistry, for the majority of adults, is paid for partly by the patient, which occurs to a much lesser extent in primary or secondary care medicine through prescription charges. The majority of the published literature focuses on changing medical practice and whilst there are likely to be many similarities between medicine and dentistry, there are also likely to be many differences (McGlone *et al.*, 2001).

Barriers and challenges have been identified to the uptake of new knowledge and research findings at various levels including at the level of the patient, the healthcare professional, the team in which they are working, the heath care organisation (for example the NHS or private sector) and also the wider environment (Grol and Grimshaw, 2003). Through the acknowledgement and identification of these challenges it may be possible to design an effective intervention that if presented in an appropriate manner will be adopted and used successfully in general dental practice (Grol and Grimshaw, 2003).

Decisions made by GDPs directly influence the oral health of the population in their care (Kay and Blinkhorn, 1996). Despite the existence of clinical practice guidelines and printed and online publications, there remains substantial variation in the clinical decisions that are made by dentists (Knutsson *et al.*, 2000; Maupomé and Sheiham, 2000). The decision-making process has evolved from planned treatment which is based around pathology to that of a greater involvement from the patient regarding choice and preference (Kay and Blinkhorn, 1996). Patients are encouraged to make an informed decision about their treatment options rather than be led by clinicians. This evolution has encouraged a two-way dialogue between practitioners and their patients, with healthcare policy in the UK driving this change (NHS, 1996; Health, 2000; NHS, 2001). The concept that clinicians always know what is best for their patients is now considered out-of-date, the most appropriate choice for a particular patient may not necessarily be the medically 'best' option (Kay and Blinkhorn, 1996; Charles *et al.*, 1999; Richards, 1999).

It has been suggested that the best decisions are those that are made following consideration of the likelihood of a successful outcome but also taking into account patients' views (Kay and Blinkhorn, 1996; Redford and Gift, 1997). The examination style, personality and ability of the dentist to relate to their patients facilitates the acceptance of treatment and engagement in decision-making (Kay and Blinkhorn, 1996; Redford and Gift, 1997). Making good decisions must, however, be underpinned by scientific evidence so that bias, however unintentional, may not be allowed to influence the decision process (Newman, 1996).

Once a diagnosis has been reached, the clinician must then decide on the most appropriate therapy that addresses the underlying pathology (Newman, 1996). Both simple and complex treatments are usually planned based upon a patient's general health, dental experience, current disease status as well as some evaluation of behaviour and emotional condition (Heinikainen *et al.*, 2002). Some treatment strategies may carry risk of failure or morbidity or higher cost and it is the role of the practitioner to consider these trade-offs whilst ensuring that the treatment provided is predictable, has a strong chance of clinical success and will not harm the patient (Newman, 1996). Dental practitioners will often use their

own clinical experience to develop their own treatment strategies, they do so without the benefit of controlled or blinded observations that often take place in clinical research trials that cannot take place in routine clinical practice (Newman, 1996). Some compromises may, however, be inevitable particularly where there are financial constraints leading to the provision of treatment that may not be the 'ideal' option (Kay *et al.*, 1995; Kay and Blinkhorn, 1996).

The published literature is a resource to which clinicians may refer to access information on evidenced-based, peer-reviewed best practice (Newman, 1996). The translation of clinical research findings into practice in dentistry, however, has been described as being 'slow, unpredictable and incomplete' (Grol and Grimshaw, 1999; Flodgren *et al.*, 2011b). The existence of published, peer-reviewed, printed educational material does not necessarily lead to an improvement in patient care and practice (Freemantle *et al.*, 1997). There is little evidence to suggest that printed material, despite the increasing volume of published material now available, results in any substantial changes in clinical practice (Freemantle *et al.*, 1997). There is also little information on how cost-effective the publication of printed material is if the hope is to elicit change in clinical practice (Freemantle *et al.*, 1997).

Many general dental practitioners may have limited access to publications in a specific field of interest. Access to articles from other disciplines many not be possible without considerable expense. Larger databases may be searched and abstracts viewed but only older articles are freely available with complete text. This cost may be prohibitive for some who access these resources rarely or do not have affiliations with secondary care organisations or higher education establishments. Reviews and systematic reviews have looked at the evidence base for the effectiveness of distributing educational materials and have shown that the evidence base for clinical practice may be changed on the basis of printed material alone (Cohen and Dacanay, 1992; Oxman *et al.*, 1995; Freemantle *et al.*, 1997; Gill *et al.*, 1999; Hulscher *et al.*, 1999).

Up-to-date knowledge of treatment options and strategies will undoubtedly influence clinical decision-making, but the way in which the knowledge was obtained may affect whether it is implemented into practice or not (Soumerai *et al.*, 1993). Scientific meetings and conferences are the obvious place for

researchers to disseminate their research findings; however the audiences for some of the more specialist meetings are likely to be other specialists within the field and may not be attended by GDPs (Newman, 1996).

Changing the decisions made by GDPs to those supported by a contemporary evidence base may improve the opportunity for achieving more predictable results and better health outcomes (Hall *et al.*, 1993; Newman, 1996). Primary care dental practitioners may be able to more readily translate the research findings into changes in clinical practice due to the substantially higher numbers of people that attend on a regular basis. Recommendations exist for practitioners to try to evaluate the evidence from dental research in a way that can be translated into their own general practice. These include examining the demographics of the cohort of patients for similarities to their own in age, type of treatment offered, general health, ethnic background, disease level, the physical surroundings and appropriate recall periods (Newman, 1996). Whilst some of these may not be fulfilled in every case, this guidance recognises that methodology of the study design should reflect every day clinical practice in determining the value of the evidence and its applicability (Newman, 1996). One difficulty that dental practitioners face is evaluating the significance of the research findings and determining whether the statistical differences found in clinical research can translate into meaningful clinical outcomes for their patients (Newman *et al.*, 2003).

Alongside publications, interactive educational meetings, multi-faceted interventions, reminders, educational outreach, audit, comments from opinion leaders, patient-mediated interventions, distributed educational materials and didactic teaching have all been the subject of a systematic review into their relative effectiveness (Bero *et al.*, 1998; York, 1999). Educational outreach is one method that has been used to achieve improvements in healthcare through the use of a trained person or team who will visit individual practitioners or practices and provide information on how a healthcare professional could modify their practice (Gurwitz *et al.*, 1990; Oxman *et al.*, 1995; Soumerai *et al.*, 1998; Hulscher *et al.*, 1999; O'Brien *et al.*, 2007). Some studies have used teams of healthcare professionals: nurses, physicians, and other professionals within a hospital environment as part of an outreach team (Hendryx *et al.*, 1998; Solomon *et al.*,

2001; Martin *et al.*, 2004). Other studies have looked at this type of intervention in a community with some specifically targeting continuing education in dentistry (Brown *et al.*, 1994). Educational outreach, particularly with a team approach, has been found to have modest effects in comparison with no intervention at all (Grimshaw *et al.*, 2004).

The use of experts or opinion leaders to disseminate information on best practice has been proposed as another method to bridge the gap between scientific research and everyday clinical practice (Flodgren *et al.*, 2011b). It is hoped that improving and optimising the method of translating evidence-based clinical practice may be achieved in this way either by individuals (Soumerai *et al.*, 1998; Leviton *et al.*, 1999; Guadagnoli *et al.*, 2000; Berner *et al.*, 2003; Sisk *et al.*, 2004) or through multidisciplinary opinion leader teams (Hong *et al.*, 1990; Elliott *et al.*, 1997; Leviton *et al.*, 1999; Cabana *et al.*, 2006; Majumdar *et al.*, 2007; Althabe *et al.*, 2008; Majumdar *et al.*, 2008; Flodgren *et al.*, 2011b). Nevertheless, the social theory suggests that the opinion leaders are perceived as being 'credible, likable and trustworthy' and are likely to be persuasive in changing behaviour and therefore established practice (Flodgren *et al.*, 2011b).

Within medicine complete uptake and implementation of national guidelines by general medical practitioners has sometimes been unsuccessful because the manner in which the guidance is published and disseminated. The passive distribution of clinical guidelines and publications has been found to have little or no effect (Bero *et al.*, 1998). Yet within the United Kingdom (UK) there is a plethora of guidance that continues to be issued from a number of sources including the National Institute of Clinical Excellence (NICE), the Scottish Intercollegiate Guidelines Network (SIGN), the Cochrane Collaboration, Royal College of Surgeons of England, national and European specialist societies, and trade unions such as the British Dental and British Medical Associations.

The contractual arrangements and remuneration that general dental practitioners receive has a direct impact on the type of treatments carried out. From 1951 until 2006 the dental contract in England and Wales was based upon individual items of treatment (Chalkley *et al.*, 2010; Hopper *et al.*, 2011). The NHS General Dental Services (GDS) contract underwent radical changes in 2006, where patients were no longer 'registered' with one practice and items of treatment were banded

together with patients being charged depending upon the band of treatment provision (Health, 2005b).

There is little high quality evidence to suggest that audit or external inspections improve compliance with standards or modify behaviour. The responsibilities of clinical audit were 'devolved' away from the individual practices and back to primary care trusts in 2001 and the financial incentives for carrying them out were also removed (Cannell, 2012). Following the Health and Social Care Act (Department of Health, 2008) all primary healthcare services are now under the scrutiny of the Care Quality Commission. It is possible that the change in regulation may have also exerted an influence on the decisions made in every day practice.

Qualitative studies have attempted to identify trends in dentists' perceptions and use of research findings (Allison and Bedos, 2003; Watt *et al.*, 2004; Dyer and Robinson, 2006; Hopper *et al.*, 2011). The most recent study to examine dentists' perceptions of research was carried out in the North West of England, in a series of qualitative research interviews. The authors concluded that more research was required to understand how best to incentivise an evidence-based culture in primary dental care (Hopper *et al.*, 2011). Other commentators have questioned the usefulness of continuing professional development and in particular validating the 'verifiability' of some sources of further education (Kelleher, 2012). If future qualitative research is to be undertaken, the consolidated criteria for reporting qualitative research (COREQ) framework should be used to help design, conduct and report qualitative research appropriately (Tong *et al.*, 2007).

Contractual and legislation changes along with the significant increase in emphasis to participate in continuing professional development may have an effect on how practitioners engage with evidence-based practice. By trying to understand some of the problems and challenges facing primary care dentists and how they engage with the ever increasing amount of published research, it may be possible to design and implement effective strategies to translate research into dental practice (Grol and Grimshaw, 2003).

1.7 Aim of the thesis

This aim of this thesis is to undertake translational studies that evaluate plaque control interventions. This will be undertaken in three parts:

1. A longitudinal clinical study, with two sequential plaque control interventions, to characterise and correlate clinical, microbial and host response parameters in subjects with mild to moderate gingivitis.

2. A randomised controlled trial examining the effect of a personalised plaque control programme for patients with oral lichen planus-attributed desquamative gingivitis.

3. A qualitative study to investigate the perceived barriers to implementing clinical research findings in general dental practice in North East England.

Chapter 2

A longitudinal study to characterise and correlate clinical, microbial and host response parameters in subjects with mild to moderate gingivitis (Clinical Trial 1)

2.1 Introduction

Periodontal diseases are inflammatory conditions which affect tooth supporting tissues and can be divided into two basic categories: gingivitis and periodontitis (Armitage, 1999). Gingivitis is a more superficial inflammation; it is confined to the gingival tissues, and is reversible with simple treatment to improve oral hygiene. Periodontitis is destructive and results in the loss of tooth attachment and bony destruction. Clinical trials usually evaluate the effectiveness of hygiene phase therapy and professional prophylaxis from a clinical, biochemical or a microbiological perspective; rarely do they correlate multiple outcomes (Hellstrom *et al.*, 1996; Bogren *et al.*, 2007; Teles *et al.*, 2007; Lalic *et al.*, 2012; Scott *et al.*, 2012). Previous interventions have assessed shifts in biomarker panels to evaluate the relationships between bacteria and their hosts in health and diseased subjects (Kumar *et al.*, 2006; Offenbacher *et al.*, 2007; Aspiras *et al.*, 2008; Barros *et al.*, 2008; Joshi *et al.*, 2008; Offenbacher *et al.*, 2010). There has, however, been a focus to try and correlate the clinical picture with both biological markers and bacterial species (Offenbacher *et al.*, 2007; Offenbacher *et al.*, 2010; Salvi *et al.*, 2010). The aim of this study is to help characterise the context in which gingivitis resolves following treatment observing clinical changes as well as those at the biochemical and microbiological level (Kumar *et al.*, 2006; Offenbacher *et al.*, 2007).

2.2 Aim

The primary aim of this study was to characterise clinical parameters with microbial and host-responses, following a formal oral hygiene programme and professional prophylaxis, in subjects with mild to moderate gingivitis. The secondary aim was to develop further understanding of hygiene phase therapy that could be utilised in further studies with more aggressive or symptomatic gingival inflammation.

2.3 Materials and methods

This study had an open-label (non-blinded) longitudinal design; all subjects enrolled in the study received the same two sequential interventions (Figure 7). The first was instruction in brushing twice daily for two minutes with a powered toothbrush, Sonicare *Flex*Care+ HX6942/20 (Philips Oral Healthcare Inc. Bothell, WA, USA). The second was a professional prophylaxis delivered by a dental hygienist. Subjects were recalled at 2-week intervals from baseline for the 8-week duration of the study. Clinical markers of health and disease were recorded and samples of gingival crevicular fluid and dental plaque taken to allow biomarker analysis.

The clinical component of the study was carried out in the Department of Periodontics at Newcastle Dental Hospital, Newcastle upon Tyne, United Kingdom. The laboratory biomarker analysis was carried out at the Cytokine Analysis Facility, University of North Carolina, Chapel Hill, North Carolina, United States and the microbiological analysis at Ohio State University, Columbus, Ohio, United States.

The study was conducted in accordance with ICH Good Clinical Practice (GCP) guidelines. A favourable ethical opinion was provided by Sunderland Research Ethics Committee (REC), United Kingdom, on 29th January 2010 (ref. 10/H0904/2). The study was sponsored by Philips Oral Healthcare, Bothell, WA, United States. Local approval was provided through Newcastle Upon Tyne NHS Foundation Trust's Research and Development Office. The study was insured by ACE Insurance and Chartis, Europe for all aspects of the study (policy numbers on file). External audit was carried out during the study by Harrison Clinical Research Limited (UK) to ensure compliance with the approved protocol.

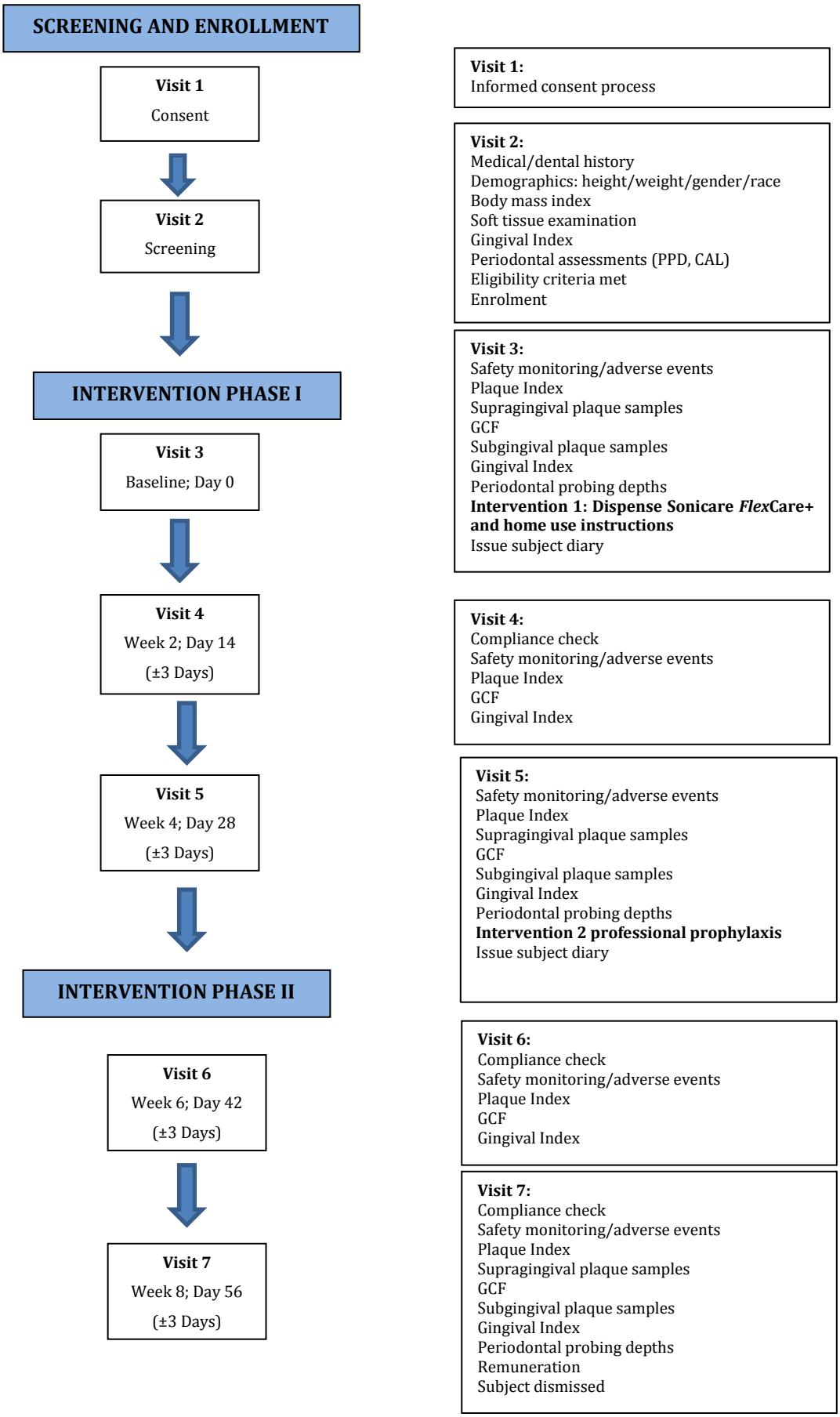


Figure 7. Clinical Trial 1. Study overview.

2.3.1 Subjects

Potential subjects were recruited from staff and students within Newcastle Dental Hospital and the School of Dental Sciences, Newcastle University, United Kingdom. Advertisements were placed around the Dental Hospital and Faculty of Medical Sciences to participate in a tooth-brushing study. Potential subjects who contacted the study co-ordinator were provided with a Research Ethics Committee (REC) approved participant information sheet detailing information to further consider their voluntary participation (Appendix 2). 31 participants were identified and further appointments were made for a screening visit, where consent was obtained and the study inclusion and exclusion criteria applied. If a subject did not meet the eligibility criteria for bleeding sites, they were withdrawn from the study.

Inclusion criteria

Subjects were included in the study if they fulfilled the following criteria:

- Adults between 18-65 years;
- In generally good health;
- Have a minimum of 20 natural teeth;
- Be a regular manual toothbrush user;
- Able to comply with study protocols and procedures and be available at all times required for participation;
- Gingival Index (Löe and Silness, 1967) ≥ 2 on at least 20 sites;
- Non-smoker.

Exclusion criteria

Subjects were excluded from the study if they met any of the following criteria:

- Infectious or systemic diseases that may be unduly affected by participation in this study;
- Diabetes mellitus;
- Insufficient capacity that may affect the participant's ability to properly follow study instructions;
- Pregnancy or nursing of infants at time of enrolment;
- Undergoing or required extensive dental, periodontal, orthodontic or implant treatment;
- A cardiac pacemaker or automatic implanted cardiac defibrillator;
- Antimicrobials within 3 weeks of enrolment in the study;
- Anti-inflammatory medication;
- Taking anti-coagulant medication;

- Had severe gingivitis defined as whole mouth average Gingival Index (Löe and Silness, 1967) of >2.5;
- Existence of periodontal pockets with concurrent attachment loss of >1.0mm at > 5 sites in any one quadrant;
- Had heavy deposits of calculus, either supragingival and/or subgingival;
- Extensive crown or bridge work and/or rampant decay at the discretion of the examiner;
- Using bleaching trays or professional whitening treatment at time of enrolment;
- Unwilling to abstain from using mouthrinses, dental floss, toothpastes (other than that provided for the project) or other toothbrushes for the duration of the study;
- Had oral surgery within 6 months of enrolment;
- Had orthodontic brackets;
- Had received a dental prophylaxis within 1 month prior to enrolment;
- Oral or extra-oral piercing with ornament or accessory in/or surrounding the oral cavity;
- Had been a participant in a dental research study within the previous 20 days prior to enrolment;
- Were a current or regular user of an electronic/power/battery toothbrush (prior periodic use or in other studies permitted) at time of enrolment;
- Were employed by a company that produces, distributes or markets dental products.

Prior and Concomitant Therapy

Subjects who were current, regular powered toothbrush users were excluded from the study. At baseline the use of any additional oral hygiene treatments or aids other than those prescribed were prohibited. These included but were not limited to: mouthwash, dental floss or other interproximal cleaning aids, chewing gum or whitening products. The use of any dentifrice was prohibited other than Colgate Cavity Protection, which was provided to subjects during the study. Any subject who was prescribed antimicrobials during the study was subsequently withdrawn. Extended, defined as over 3 days, or chronic use of anti-inflammatory or anti-coagulant medication (over the counter or prescription) at any time whilst participating in the study was also prohibited.

2.3.2 Power and sample size

The primary endpoint was the change in Gingival Index (Löe and Silness, 1967), Plaque Index (Silness and Löe, 1964) and biomarkers from baseline after introduction of an oral hygiene programme and after professional prophylaxis.

The Gingival Index (Löe and Silness, 1967) was used for the purposes of calculating a sample size.

The study was designed to have a high probability (statistical power) of detecting a moderate change in mean Gingival Index over time. The assumption was made that a standard deviation of 0.2 was a reasonable estimate of the within-group standard deviation. 30 subjects would be required to detect with 90% power a mean Gingival Index change of 0.12 or greater.

2.3.3 Clinical measures

Plaque was measured on a four-point index of Silness and Löe (Silness and Löe, 1964). This Plaque Index (PI) scores plaque on the surface of teeth without the use of a disclosing solution on a scale of 0 to 3. It is dependent upon a clinical examiner interpreting the difference between the following:

Code	Descriptor
0	No plaque.
1	A film of plaque that is only visible by running a dental probe along the gingival margin.
2	A moderate level of plaque that is visible to the naked eye.
3	An abundance of plaque on the tooth surface.

A full mouth PI at 6 sites per tooth (distobuccal, buccal, mesiobuccal, distolingual, lingual, mesiolingual) was recorded.

Inflammation of the gingival tissues was recorded using the Löe and Silness Gingival Index (GI) (Löe and Silness, 1963). Full mouth GI at 6 sites per tooth (distobuccal, buccal, mesiobuccal, distolingual, lingual, mesiolingual), recorded on a scale of 0 to 3 where:

Code	Descriptor
0	Normal gingivae.
1	Mild inflammation, slight change in colour, slight oedema, no bleeding on probing.
2	Moderate inflammation, redness, oedema and glazing, bleeding on probing.
3	Severe inflammation, marked redness and oedema, ulceration, tendency to spontaneous bleeding.

Full mouth pocket probing depth (PPD) and clinical attachment loss (CAL) were recorded at 6 sites per tooth (distobuccal, buccal, mesiobuccal, distolingual, lingual, mesiolingual), excluding third molars, and recorded in millimetres using a UNC-15 probe (Hu-Friedy Manufacturing Co., Inc. Chicago, IL, USA).

Biomarkers

Gingival Crevicular Fluid (GCF) samples were collected and the volume recorded from each subject at specific intervals (visits 3-7) to determine local levels of inflammatory biomarkers. The biomarkers analysed were: IL-1α, IL-1β, IL-8, MIP-1α, MIP-1β, RANTES, MMP-1, MMP-3, MMP-8, MMP-9, MMP-13. These were chosen because they are thought to mediate phases of the inflammatory response in periodontal disease, and are produced in response to the presence of an active biofilm and its products. Supragingival and subgingival plaque samples were collected at visits 3, 5 and 7 to determine levels of pre-selected microorganisms.

2.3.4 Examiner calibration

In order to minimise bias, and ensure the validity of any conclusions drawn from the results of the study, a calibration exercise was undertaken on three clinical dental parameters: Plaque Index (PI), Gingival Index (GI), and Probing Depth (PD). Repeat measures were performed on subjects not involved in the main study. An additional examiner was also calibrated in the event that the primary examiner was unable to carry out the clinical examinations.

It was appropriate to employ a weighted Cohen's Kappa statistic to assess the agreement between two raters after adjusting for chance (Cohen, 1968). The use of weighting ensures that a disagreement of more than one unit arising between replicate 1 and replicate 2 is more heavily penalised than a disagreement of only one unit. The weighted Cohen's Kappa for three clinical parameters was: PI = 0.80 [95% CI 0.75, 0.84]; GI = 0.77 [95% CI 0.70, 0.82]; PD = 0.84 [95% CI 0.78, 0.82]. Although the guidance for interpreting the weighted Kappa statistic results varies, agreements above 0.7 are taken as implying very good-to-excellent agreement (Cohen, 1968; Viera and Garrett, 2005).

2.3.5 Study visits

Subjects were asked to attend for a total of 7 study visits over 8 weeks, with visits 1 and 2 permitted to be on the same day (Figure 7).

Visit 1 Subject information and consent

Written informed consent was obtained from each subject at the screening visit prior to enrolment and the initiation of any study related procedures (Appendix 3). Potential subjects were scheduled for a screening appointment and provided with a REC approved information leaflet and consent form to read. They were then given the opportunity to ask any relevant questions about the study. If they agreed to participate they signed the consent form and were offered a copy for their records. If requested, subjects were given a further period of time (up to 24 hours) to consider their willingness to take part in the study. The research team or Principal Investigator answered any questions that were raised by potential participants. Original copies of the signed informed consent forms were retained on file at Newcastle University.

Visit 2 Screening

Those subjects who wished to participate were screened for their eligibility. They were instructed to brush their teeth 2-6 hours before the next appointment. A medical history was recorded and was reviewed by the investigator to assess eligibility. Demographics (date of birth, age, gender, race, height, weight, BMI) were recorded for each subject.

An intraoral soft tissue examination was performed noting particularly any gingival abrasions, irritation, lacerations or ulceration. The GI was recorded at 6 sites per tooth. Subjects required a mean GI of at least 2.0 over at least 20 sites to continue in the study. Full mouth periodontal probing depths were recorded using a UNC-15 periodontal probe at 6 sites per tooth rounded to the next lower whole millimetre. Subjects with periodontitis, defined as recording periodontal pockets with concurrent loss of attachment greater than 1.0mm at greater than 5 sites in any one quadrant, were excluded. Clinical attachment loss (CAL) was measured using a UNC-15 periodontal probe at 6 sites per tooth to measure the distance in millimetres from the cementoenamel junction (CEJ) to the base of the gingival sulcus. Subjects who fulfilled all of the inclusion/exclusion criteria were then scheduled for baseline assessment.

Visit 3 Baseline

Visit 3 took place within 14 days of visit 1. As with visit 2, subjects had been instructed to brush their teeth within 2-6 hours prior to the appointment. The subjects' medical and dental histories were reviewed for any adverse events that may be attributed to participation in the study. An intra-oral soft tissue examination and full mouth PI was performed. GCF, supra- and sub-gingival plaque samples were collected from 2 sites per quadrant (Figure 8). A full mouth GI was then recorded followed by full mouth periodontal pocket depths as described in Visit 2.

The first of the two interventions (hygiene phase therapy) was provided at visit 3. All subjects received a Sonicare *Flex*Care HX6942/20 with ProResults brush head (Philips Oral Healthcare Inc. Bothell, WA, USA). They were given comprehensive written and verbal instructions to brush their teeth twice daily for 2 minutes using standardised toothpaste Colgate Cavity Protection (Colgate-Palmolive, Guildford, Surrey, UK; active ingredient sodium monofluorophosphate 0.76%). The Sonicare *FlexCare* toothbrush did not have the 'easy start' feature active and was used in its default-brushing mode 'clean'.

Subjects were asked to keep a brushing diary to aid with compliance and to brush 2-6 hours prior to the next appointment. No other interdental cleaning aids, mouthrinses or chewing gum were permitted during the study. Subsequent appointments were then scheduled for the remaining study visits.

Visit 4

Visit 4 took place 14 days after Visit 3 (+/- 3 days). A compliance check was carried out with a verbal interview and review of the brushing diary to determine if the subject had followed the study instructions. Soft tissue examination, medical and dental histories were recorded along with any reportable adverse events. A full mouth PI was carried out prior to the collection of GCF at the same sites described in Visit 3. A full mouth GI was then carried out; no plaque samples were collected at this visit.

Visit 5

Visit 5 took place 28 days after Visit 3 (+/- 3 days). A compliance check was carried out with a verbal interview and review of the brushing diary. Soft tissue

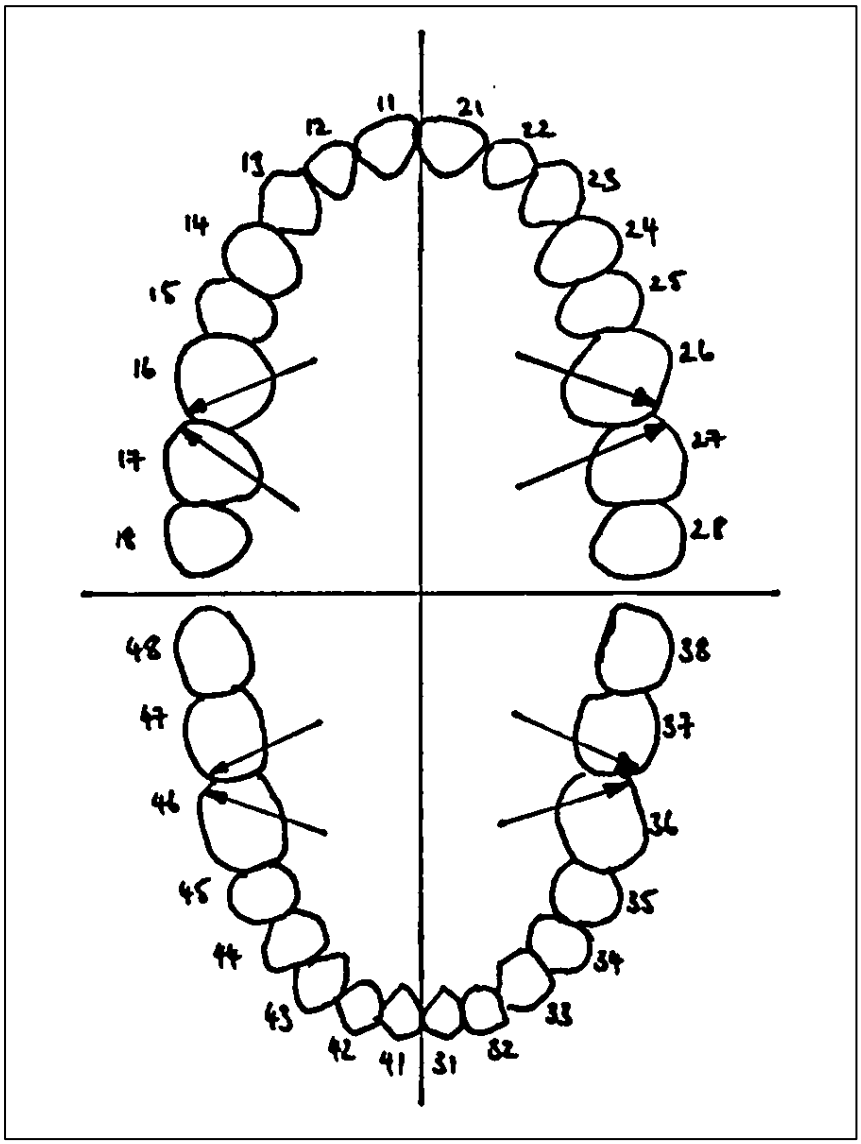


Figure 8. Clinical Trial 1. Sites sampled for plaque and GCF.
All four quadrants were sampled using buccal surfaces from the mesial of the second molar (171, 271, 371, 471) and the distal of the first molar (163, 263, 363, 463).

examination, medical and dental histories were recorded along with any reportable adverse events. Clinical records of PI, GI, PPDs along with supra and subgingival plaque samples and GCF were recorded as previously described. A new brushing diary was also issued.

The second of the two interventions was provided at this visit. A full mouth prophylaxis was undertaken by a dental hygienist, this included scaling to remove supra and subgingival calculus deposits and a full mouth prophylaxis (Prophylaxis paste, Kemdent®, UK).

Visit 6

Visit 6 took place 42 days after Visit 3 (+/- 3 days). A compliance check was carried out with a verbal interview and review of the brushing diary. Soft tissue examination, medical and dental histories were recorded along with any reportable adverse events. A full mouth PI was carried out prior to the collection of GCF at the same sites as described in Visit 3. A full mouth GI was then carried out; no plaque samples were collected at this visit.

Visit 7

Visit 7 took place 56 days after Visit 3 (+/- 7 days). A compliance check was carried out with a verbal interview and collection of the brushing diary. Soft tissue examination, medical and dental histories were recorded along with any reportable adverse events. Clinical records of PI, GI, PPDs along with supra and subgingival plaque samples and GCF were recorded as previously described. Subjects were compensated for participation in the study with £70 in gift vouchers before being dismissed from the study.

2.3.6 Biomarker collection

Gingival crevicular fluid samples

GCF was collected at 2 weekly intervals from baseline (Visits 3,4,5,6 and 7) using the following method: GCF was collected using Periopaper® collection strips (Oraflow, Smithtown, NY, USA) which were inserted gently into the gingival crevice. The teeth were air dried and isolated with cotton rolls prior to placement of the Periopaper® strips to reduce saliva contamination. Sufficient pressure was applied to allow the Periopaper® to be held just within the gingival crevice. The Periopaper® was left in place for 30-60 seconds or until the paper visibly

dampened at the lower third. The paper was then removed and the volume determined using a calibrated Periotron 8000 (Oraflow, Smithtown, NY, USA). This device had been allowed to warm up for at least 10 minutes prior to use. In addition to the warm up time, dry Periopaper strips were used to adjust the reading on the Periotron to "0." Two consecutive readings of zero were determined with a reading between 002 and -002 being acceptable tolerances. After measuring the volumes of GCF the metal contacts of the Periotron were cleaned with alcohol and dry gauze to prevent cross contamination of samples and residual moisture affecting subsequent readings.

Calibration of the Periotron was carried out according to the manufacturer's protocol to determine the relationship between periotron readings and standard distilled water volumes (Oraflow, 2007). The calibration data were then used to determine the concentrations of mediators in each of the samples of GCF taken from the subjects during analysis. Comprehensive calibrations (12 volumes repeated three times) of the Periotron were carried out monthly for the duration of the study with smaller interim calibrations taking place weekly. Records of the interim and comprehensive calibrations were retained in the main study file.

GCF samples were taken from 2 sites per quadrant with the preferential sites being the mesiobuccal site of the second permanent molar and the distobuccal site of the first permanent molar. If these teeth were absent then the sample was taken from the next available mesial tooth. The quadrant tooth site (QTS) notation was used for site nomenclature and identification with the same sites being used for both GCF and plaque collection for microbial assessment: 171/163, 271/263, 371/363, 471/463 (Figure 8).

Plaque samples

Supragingival plaque was collected from the target sites by sweeping a curette or scaler at the first site and wiping onto 2 paper points. Subgingival plaque samples were collected by inserting 5 paper points into the gingival sulcus of the site for 10 seconds. Following removal of the paper points, the sulcus was swept with a scaler or curette and the plaque wiped onto the same points. The paper points (Dentsply Maillefer, Ballaigues, Switzerland) were then placed into the prelabelled cryovial and the procedure repeated for the subsequent sites in the remaining quadrants. Samples were placed into barcoded (for subject number, site and visit) 2mL

Eppendorf microcentrifuge tubes and placed in a freezer at -80°C until shipment to Ohio State University, OH, USA for analysis.

2.3.7 Biomarker analysis

GCF analysis

Analysis of the GCF samples took place at the University of North Carolina (UNC) Cytokine Analysis Facility, Chapel Hill, NC, USA. The protocols for multiplex cytokine analysis have previously been published using the R&D Systems Fluorokine® MultiAnalyte Profiling kits (Offenbacher *et al.*, 2007; Offenbacher *et al.*, 2010). Samples were collected onto filter paper strips (Periopaper®, Ora*flow,* Smithtown, NY, USA) and the volume determined using a Periotron 8000® as previously described (Oraflow, Smithtown, NY, USA).

Following determination of the GCF volumes, the samples were then wrapped in autoclaved aluminium foil and placed into two barcoded 2mL Eppendorf microfuge tubes. The sample tubes were pre-labelled with unique identifiers for the study and specimen. Samples were then flash frozen in liquid nitrogen (stored in a nearby Dewar flask) and placed in a temperature-controlled freezer (-80°C) until shipment on dry ice to UNC and then stored in liquid nitrogen (-180°C) until mediator analysis.

Sample preparation

Two samples per subject visit (463,471) were prepared and analysed separately for the following mediators IL-1α, IL-1β, IL-8, MIP-1α, MIP1β, RANTES, MMP-1, MMP-3, MMP-8, MMP-9, MMP-13. Analysis was performed to quantify the levels of these inflammatory mediators using the Fluorokine® Profiling MAP cytokine multiplex kits (R&D Systems, MN, USA) and the Bio-Plex® 200 analyser system (Luminex Corporation, DeSoto, TX, USA) (Figure 9). The cytokines (IL-1α, IL-1β, IL-2, MIP-1α, MIP-1β) were analysed using Fluorokine® MAP multi analyte profiling human base kit whereas the matrix metalloproteinases (MMP-1, MMP-3, MMP-8, MMP-9, MMP-13) were analysed using human MMP base kit (R&D systems, MN, USA).

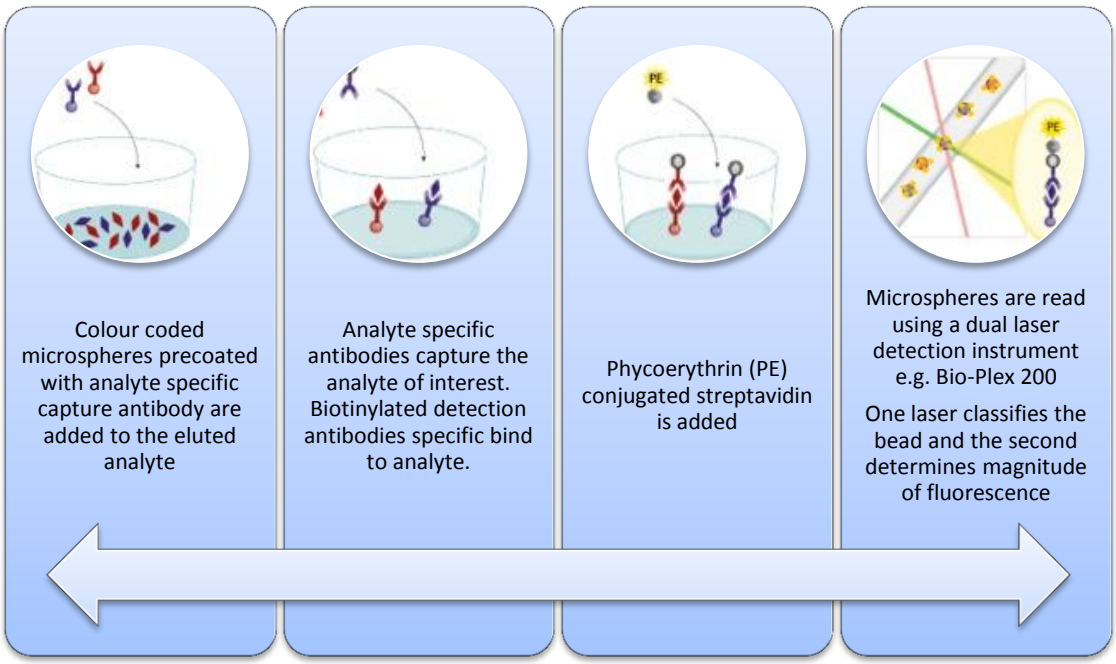


Figure 9. Clinical Trial 1. Overview of the principle of the Bio-Plex® 200 multiplex assay (R&D Systems, MN, USA).

The GCF samples were eluted according to Luminex Corporation protocols for each of the base kits for human MMP and cytokines (R&D systems, MN, USA). The samples were thawed and the Periopaper® strips eluted with 200 μL phosphate buffered saline (PBS) into a 96-well vacuum manifold collection plate. They were then placed on a microplate shaker at 450 rpm for 30 minutes at room temperature. The working standard curve, which allowed comparison with the samples, was made up. 100 μL of the two standard cocktails of recombinant human cytokines in each base kit (R&D systems, MN, USA) were mixed then agitated, using a Vortex-Genie 2 mixer (Scientific Industries, Bohemia, NY, USA) for 30 seconds. The standard mixture was then diluted in serial as per the standard value cards supplied in the Fluorokine® MAP base kits. Following dilution the standards were gently agitated at 450 rpm (VWR Signature™ Microplate Shaker, Radnor, PA, USA) for 15-30 minutes.

Analyte specific antibodies (to e.g. MMP-1) were pre-coated onto colour-coded microspheres supplied by the manufacturer in the form of a concentrate. The concentrates were vortexed to resuspend the microspheres; 50 μL of each microparticle concentrate was added to a mixing bottle with 5 mL of microparticle diluent before vortexing the mixture.

The microplate was pre-wetted with 100 μL wash buffer to stop the microspheres clumping together and errors occuring during analysis, the liquid was removed using a vacuum manifold designed to accommodate the microplate.

The first two vertical columns of the plate were used for the serial dilution standards. 50 μL of the microparticle mixture was then added to the microplate along with 50 μL of sample or standard. The plate was sealed and incubated for 3 hours at room temperature on a microplate shaker at 450 rpm; the layout of the plate was recorded to record standards and samples to be assayed.

The plate was then washed by removing the liquid with the vacuum manifold. This was done by filling each well with 100 μL wash buffer then removing the liquid again this process was repeated three times. The biotin detection antibodies were prepared by centrifuging the antibody vial for 30 seconds followed by re-suspending the concentrate using a vortex. 50 μL of biotin antibody concentrate was then added to a vial of biotin antibody diluent and gently mixed. 50 μL of

diluted antibody cocktail was added to each well of the microplate then covered and incubated for a further hour on a microplate shaker at 450 rpm.

The streptavidin-phycoerythrin conjugate (Strepavidin-PE) was prepared in an amber polylpropylene bottle to prevent light contamination. It was diluted by 100 times by adding 55 μL of Strepavidin-PE to 5.5 mL of wash buffer. 50 μL of diluted Strepavidin-PE was added to each well of the microplate, covered with a foil plate sealer and incubated for 60 minutes on a microplate shaker at 450 rpm. Following a final wash with wash buffer to remove unbound Streptavidin-PE, the microspheres were re-suspended in 100 μL of wash buffer in each well and agitated for 2 minutes on the microplate shaker at 450 rpm.

Detection

The plate was then read using the Bio-Plex® 200 analyser; the microspheres were drawn up from the wells and read using two lasers. The first identifies the bead type and determines the analyte being detected. The second laser determines the magnitude of the fluorescence signal, which is in direct proportion to the amount of bound analyte. All biomarker mediator values were then corrected for elution volume, assay dilution and GCF volume and expressed as a GCF concentration.

Microbial analysis

Analysis of the plaque samples was carried out at the College of Dentistry, Ohio State University, OH. Analysis was carried out on pooled samples from one quadrant for each subject for each visit, as has previously been described (Shchipkova *et al.*, 2010). The Applied BioCode System (Applied Biocode Inc., CA, USA) has not been widely used for determination of oral bacteria, but the underlying chemistry is similar to the immunoassays performed with the GCF samples. It uses small (25μm x 75μm x 5μm) carboxyl barcoded magnetic beads (BMBs) to simultaneously detect up to 32 different analytes within a single sample. The molecular reaction takes place on the surface of digital barcoded magnetic beads (Figure 10). Target specific capture probes are covalently linked to a specific set of digital barcoded beads. Biotin-labelled targets (single stranded DNA) are captured by the bead bound capture probes in a hybridisation suspension. Streptavidin-phycoerythrin conjugate (SA-PE) is finally added to the samples to hybridise the targets and allow detection (Applied Biocode, 2013a).

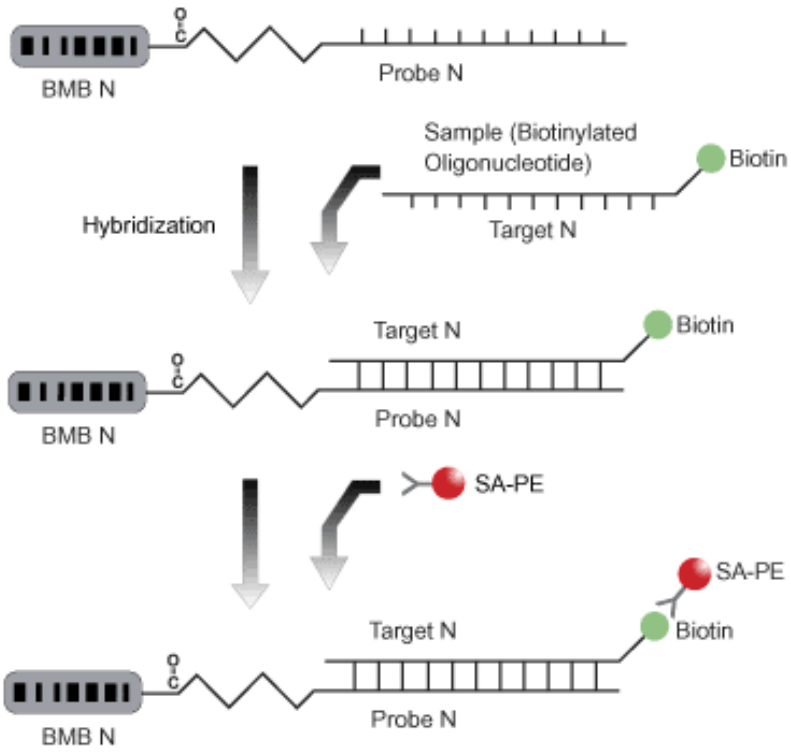


Figure 10. Clinical Trial 1. Molecular bioassay protocol using Barcoded Magnetic Bead (BMB) Multiplex technology.
The barcoded magnetic bead is formed by combining a biocompatible polymer with paramagnetic material, this allows for highly stable surface chemistry and the magnetic properties allow easy handling and washing of the beads when an external magnetic force is applied. The carboxyl BMBs allow DNA probes for specific bacteria to bind the bead surface (Applied Biocode, 2013a).

Sample preparation

200 µL phosphate-buffered-saline (PBS) were used to elute the bacteria from the paper points in the microcentrifuge tubes. The samples were agitated at 450 rpm using a microplate shaker for 2 hours at room temperature followed by centrifuging. The paper points were removed and the bacterial DNA was isolated with a Qiagen DNA MiniAmp kit (Qiagen, Valencia, CA, USA) using the tissue protocol according to the manufacturer's instructions. The bacterial DNA was amplified using Polymerase Chain Reaction (PCR) and labelled using A17 (5' GTT TGA TC TGG CTC AG 3') and 317 (5' AAG GAG GTG ATC CAG GC 3') primers (Biosythesis, Lewisville, TX, USA) as previously described (Kumar *et al.*, 2005).

The products of the PCR reaction were then purified to remove impurities from the samples (e.g. primers, enzymes, mineral oils, salts) and pure DNA was eluted with small volumes of endotoxin specific (ES) buffer. A Qiaquick spin column (Qiagen, Valencia, CA, USA) was placed into a conventional 2mL collection tube. The sample was added to the column and centrifuged for 30-60 seconds at 13,000 RPM in a conventional table-top microfuge at room temperature. The flow through was discarded and the column was placed back into the same tube and washed with 0.75mL PE buffer before being returned to the centrifuge for a further 30-60 seconds. Once the residual wash buffer was removed, the DNA was eluted in 50 µL of elution buffer and centrifuged for a further 1 minute. Agarose gel electrophoresis was used to confirm successful amplification of the bacterial DNA to confirm validity of the samples as PCR can be sensitive to contamination.

Barcoded magnetic bead- probe coupling and hybridisation

The bacteria specific capture probes were then covalently coupled to the carboxyl barcoded magnetic beads as described in the manufacturer's protocol. Following this the beads were re-suspended in 200 µL PBS-T and stored at 2-8 °C prior to hybridisation with the sample (Applied Biocode, 2013b). The bead-bound probes were then hybridised to the biotin labelled PCR product prior to the addition of Streptavidin-R-Phycoerythrin and detection buffer to hybridise the target and allow detection of the sample.

Detection

A 96 well microplate was placed into the BioCode 1000 analyser (Applied Biocode Inc., CA, USA) for detection. A bright LED with a charged couple device (CCD)

camera to illuminate the beads was used to obtain a barcode image. Secondarily the LED was used to obtain a fluorescent image. The images were then decoded for each individual bead and the amount of fluorescence intensity, which directly related to the microbial levels in the sample.

2.4 Data management and confidentiality

Study records, including each volunteer's signed informed consent and other study related documents were kept in a secure area under the supervision of the Principal Investigator. All study data (excluding laboratory data) were recorded on paper based clinical record forms. Data were double-entered into an electronic, web-based data system (InForm Version 4.6) provided by the study sponsor, Philips Oral Healthcare. All personal subject identifiers were removed from the data set and all enrolled subjects were assigned a specific study number. Only the investigators at Newcastle University had access to information linking the subjects to the corresponding assigned study number.

Access to the electronic data entry system was protected by login identification and passwords. Data entry as well as all data modification, was documented by the system and available in an audit trail. De-identified study data were also retained by the study sponsor. Paper based records of enrolment metrics, safety information and concomitant medication were retained on file at Newcastle University.

During data collection any modification to a written form or document was amended with a single line through the erroneous data. The correction was legibly entered as well as the initials and date of the person making the correction. The master list of participants was retained in the event that subjects needed to be contacted after study completion. After the conclusion of the clinical part of the study, electronic clinical record forms was completed and digitally signed, the clinical database was then electronically locked.

2.5 Statistical and analytical plan

The population to be analysed comprised all enrolled subjects with available post-baseline data. Analysis were conducted using SAS/STAT® software. Continuous variables were summarised using descriptive statistics: number of obervations, mean, standard deviation, median minimum and maximum. The Plaque Index, Gingival Index and Pocket Probing Depths were analysed using analysis of variance (ANOVA), the following model was used:

$$y_{ij} = \mu + \alpha_i + W_j + \varepsilon_{ij}$$

Where:

y_{ij} Change from baseline for each subject ($_i$) at week ($_j$) (2,4,6,8);

μ overall effect;

W_j extent to which change from baseline is attributable to week ($_j$);

α_i random subject effect;

ε_{ij} unexplained/residual variation for each subject ($_i$) at week ($_j$) (2,4,6,8).

The biomarker data were summarised using descriptive statistics for the observed values and the changes from baseline. Two sided t-tests, without adjustment for multiple comparisons were used to assess the statistical significance of changes from baseline.

2.6 Results

A total of 31 subjects were screened. All fulfilled the entry criteria and were consented to participate. 31 subjects were retained through the 7 visit protocol (217 study visits) to the completion of the study with no early terminations. The subjects were mostly female (67%) and white (83.9%). The mean age was 27 years. A summary of demographics is listed in Table 6.

During the study there were 13 protocol deviations, 9 of which were due to the lack of availability of liquid nitrogen to snap freeze the GCF samples. These samples were placed directly into the -80°C freezer. The deviations were not expected to have a major impact on the overall study conclusions.

Demographic		
Age (years)	Mean (SD)	27 (10.3)
	Median	21
	Min, Max	20, 54
Gender (number of subjects)	Female	21
	Male	10
Race	Asian	1
	Black	1
	White	26
	Mixed	2
	Chinese	1
Height (cm)	Mean (SD)	169.7 (9.2)
	Median	168
	Min, Max	154, 186
Weight (Kg)	Mean (SD)	68.3 (13.6)
	Median	65
	Min, Max	48, 102
BMI	Mean (SD)	23.6 (3.4)
	Median	22.4
	Min, Max	18.6, 32.6
Smoking status	Not current	31
	Never	27
	Former	4

Table 6. Clinical Trial 1. Study demographics for the 31 participants.

2.6.1 Plaque Index

The overall mean PI was calculated as an arithmetic average of all PI scores recorded at 6 sites per tooth as detailed in the protocol. Descriptive statistics are shown for PI in Table 7.

4 weeks following Intervention 1, the reduction in PI from baseline with 95% CI was 0.56 (0.43, 0.69). 8 weeks following Intervention 1 and 4 weeks following Intervention 2 the mean reduction in PI with 95% CI was 0.7 (0.6, 0.9). Analysis of variance (ANOVA) statistics (Table 8) examined the statistical difference from baseline to weeks 2,4,6 and 8 which indicated a significant reduction in PI from baseline at all weeks (ANOVA $p<0.0001$). There was a consistent reduction in PI from baseline following each intervention which was sustained to the completion of the study.

The combined effect of both interventions (PI reduction=0.70) was greater than that of one single intervention alone; a post hoc analysis which used the week 4 (Intervention 1) observation as the baseline for Intervention 2 suggests that Intervention 2 has a smaller reduction in PI compared to intervention 1 ($p=0.0005$). All of the subjects received the same two sequential interventions in the same order; it was not possible to examine whether the same effect would have been observed had the professional prophylaxis been the first intervention.

Box plots for change from baseline in overall PI by visit were generated and are presented in Figure 11. A line graph shows the reduction in mean plaque at each visit (Figure 12). Reference plots were produced that provide a visual representation of in mean PI change from baseline at weeks 4 (Figure 13) and 8 weeks (Figure 14).

Visit	n	Mean PI (SD)	Median PI	Min, Max	95% CI
Baseline	31	1.1 (0.4)	1.1	0.3, 1.8	(1.0, 1.2)
Week 2	31	0.7 (0.4)	0.8	0.1, 1.4	(0.6, 0.9)
Week 4	31	0.6 (0.3)	0.7	0.1, 1.2	(0.5, 0.8)
Week 6	31	0.4 (0.3)	0.3	0.0, 0.9	(0.3, 0.5)
Week 8	31	0.4 (0.2)	0.3	0.0, 0.8	(0.3, 0.4)
Reduction					
Baseline to Week 2	31	0.4 (0.3)	0.4	-0.0, 1.1	(0.3, 0.5)
Baseline to Week 4	31	0.5 (0.3)	0.4	-0.0, 1.1	(0.3, 0.6)
Baseline to Week 6	31	0.7 (0.3)	0.7	0.1, 1.3	(0.6, 0.8)
Baseline to Week 8	31	0.7 (0.3)	0.7	0.2, 1.3	(0.6, 0.9)

Table 7. Clinical Trial 1. Descriptive statistics for Plaque Index (Silness and Löe, 1964) including reduction in Plaque Index from baseline.

Visit/ Contrast	n	Least squares mean reduction in PI (SE)	95% CI	ANOVA p-value
Week 2	31	0.56 (0.07)	(0.43, 0.69)	
Week 4	31	0.64 (0.07)	(0.51, 0.78)	
Week 6	31	0.87 (0.07)	(0.74, 1.00)	
Week 8	31	0.94 (0.07)	(0.81, 1.07)	<0.0001
Intervention 2 (Weeks 6 and 8) vs Intervention 1 (Weeks 2 and 4)	31	0.30 (0.05)	(0.20, 0.40)	

Table 8. Clinical Trial 1. Analysis of variance (ANOVA) statistics for Plaque Index (Silness and Löe, 1964) outlining reduction in PI from baseline.

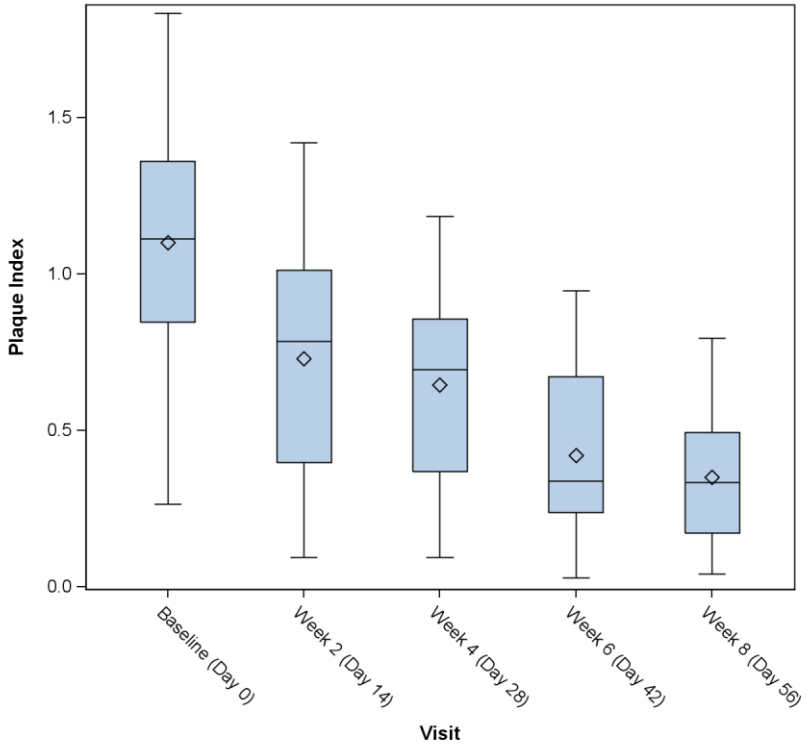


Figure 11. Clinical Trial 1. Box plot for Plaque Index over time; interventions after clinical examinations at baseline and week 4. Mean values are represented by diamonds and medians by horizontal line though the box plot, error bars are also shown. Statistical significant reductions were found at weeks 2,4,6 and 8 (ANOVA P<0.0001).

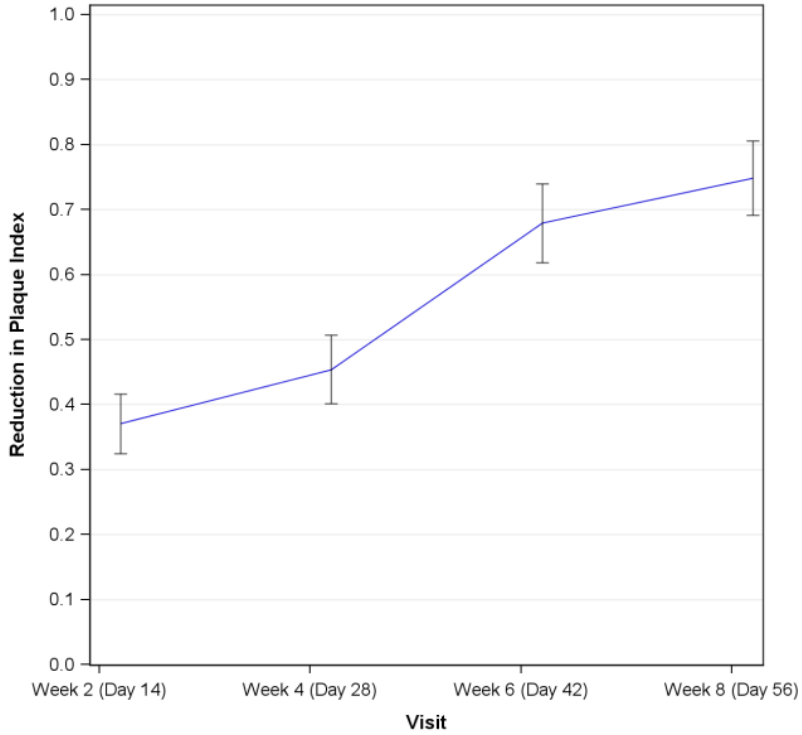


Figure 12. Clinical Trial 1. Line graph to show reduction in Plaque Index over time study with corresponding error bars. Interventions took place at baseline (day = 0) and 4 weeks. Statistical significant reductions were found at weeks 2,4,6 and 8 (ANOVA P<0.0001).

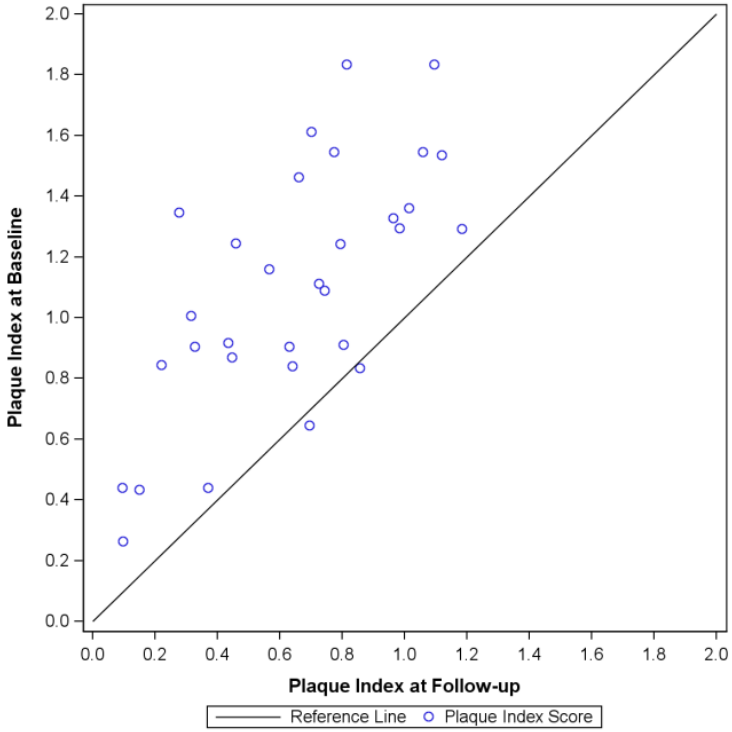


Figure 13. Clinical Trial 1. Reference plot for Plaque Index at week 4 against baseline.
Mean Plaque Index scores plotted above the diagonal line represent subjects that have improved following hygiene phase therapy.

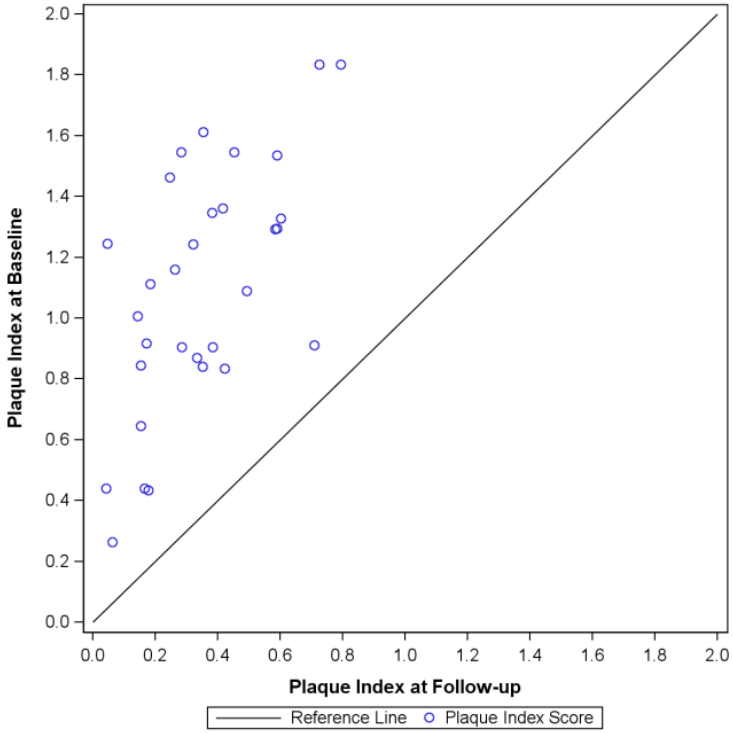


Figure 14. Clinical Trial 1. Reference plot for Plaque Index at week 8 against baseline.
Mean Plaque Index scores plotted above the diagonal line represent subjects that have improved from Baseline to follow-up, these scores represent the combined effects of Intervention 1 and Intervention 2. There are no scores below the line to indicate deterioration in Plaque Index scores from baseline for any subject.

2.6.2 Gingival Index

The overall mean GI was calculated as an arithmetic mean of all GI scores recorded at 6 sites per tooth as detailed in the protocol. Descriptive statistics are provided for GI in Table 9.

4 weeks following Intervention 1 the mean reduction in GI from baseline with 95% CI was 0.30 (0.24,0.35). Eight weeks following Intervention 1 and four weeks following Intervention 2, the mean reduction in GI from baseline with 95% CI was 0.51 (0.46,0.56). An analysis of variance (ANOVA) was carried out (Table 10) to examine the statistical difference from baseline to weeks 2, 4, 6 and 8 which indicated a significant reduction in GI from baseline at all weeks (ANOVA $p<0.0001$). There was no statistical significance in GI between weeks 6 and 8; the 95% CI included 0 and the mean reduction was 0.02 (-0.06,0.10). The results demonstrate a consistent reduction in GI from baseline following each intervention which is sustained to the completion of the study.

The combined effect of both interventions (mean GI reduction = 0.51) was greater than that of Intervention 1 alone. Intervention 2 did not have a greater overall reduction in GI compared with Intervention 1 where week 4 was used as the baseline for Intervention 2 ($p=0.2214$).

A line graph showing the reduction in GI from baseline is shown in Figure 15 , Box plots for change from baseline by visit are presented in Figure 16. Reference plots provide a visual representation of in mean GI change from baseline at weeks 4 (Figure 17) and 8 weeks (Figure 18).

Visit	n	Mean GI (SD)	Median GI	Min, Max	95% CI
Baseline	31	1.0 (0.2)	1.0	0.6, 1.3	(0.9, 1.0)
Week 2	31	0.8 (0.2)	0.8	0.4, 1.2	(0.7, 0.9)
Week 4	31	0.7 (0.2)	0.7	0.4, 1.0	(0.6, 0.7)
Week 6	31	0.5 (0.2)	0.5	0.2, 1.0	(0.4, 0.5)
Week 8	31	0.4 (0.1)	0.4	0.2, 0.8	(0.4, 0.5)
Reduction					
Baseline to Week 2	31	0.2 (0.2)	0.2	-0.1, 0.4	(0.1, 0.2)
Baseline to Week 4	31	0.3 (0.1)	0.3	0.0, 0.6	(0.2, 0.3)
Baseline to Week 6	31	0.5 (0.2)	0.5	0.1, 0.9	(0.4, 0.6)
Baseline to Week 8	31	0.5 (0.1)	0.5	0.2, 0.8	(0.5, 0.6)

Table 9. Clinical Trial 1. Descriptive statistics for Gingival Index (Löe and Silness, 1967) including reduction from baseline.

Visit/ Contrast	n	Least square mean reduction in GI (SE)	95% CI	ANOVA p-value
Week 2 (Day 14)	31	0.17 (0.03)	0.11, 0.22	<.0001
Week 4 (Day 28)	31	0.30 (0.03)	0.24, 0.35	
Week 6 (Day 42)	31	0.49 (0.03)	0.43, 0.54	
Week 8 (Day 56)	31	0.51 (0.03)	0.46, 0.56	
Intervention 2 (Weeks 6 and 8) vs Intervention 1 (Weeks 2 and 4)	31	0.27 (0.03)	0.21, 0.32	

Table 10. Clinical Trial 1. Analysis of variance (ANOVA) for Gingival Index (Löe and Silness, 1967).

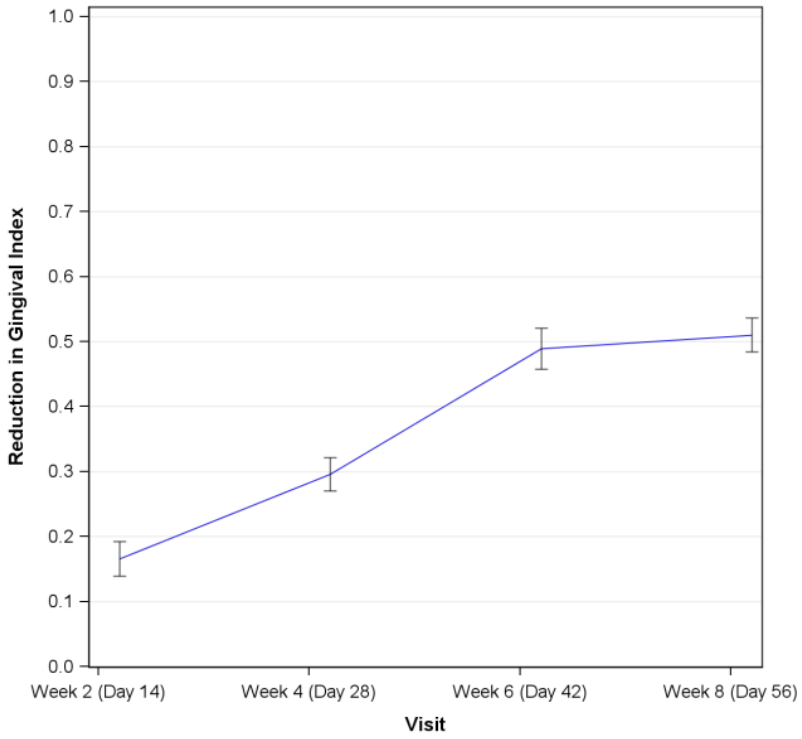


Figure 15. Clinical Trial 1. Line graph to show reduction in Gingival Index.
Statistical significant reductions were found at weeks 2,4,6 and 8 (ANOVA P<0.0001).

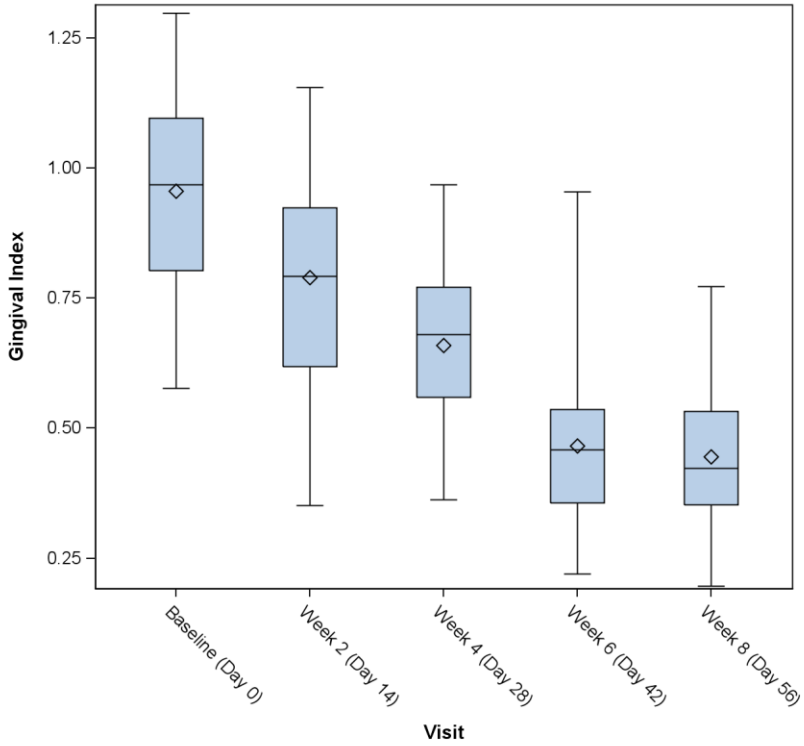


Figure 16. Clinical Trial 1. Box plot for Gingival Index over time: Interventions after the measurements at baseline (Intervention 1) and week 4 (Intervention 2).
Mean values are represented by diamonds and medians by horizontal line though the box plot, error bars are also shown. Statistical significant reductions were found at weeks 2,4,6 and 8 (ANOVA P<0.0001).

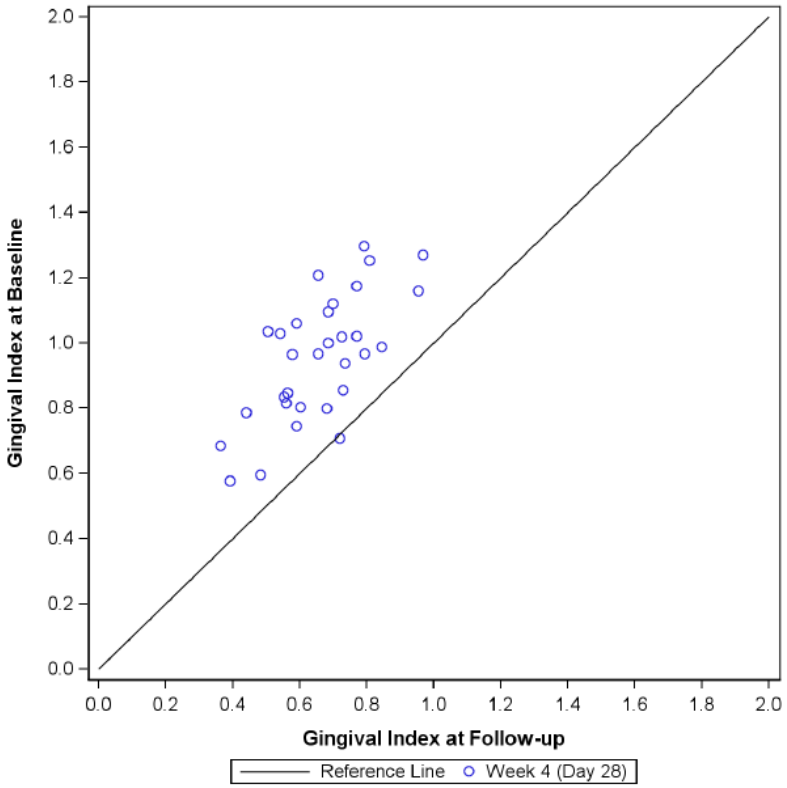


Figure 17. Clinical Trial 1. Reference plot for Gingival Index at week 4 against baseline.
Mean Gingival Index scores plotted above the diagonal line represent subjects that have improved from Baseline to follow up 4 weeks after Intervention 1 (hygiene phase therapy).

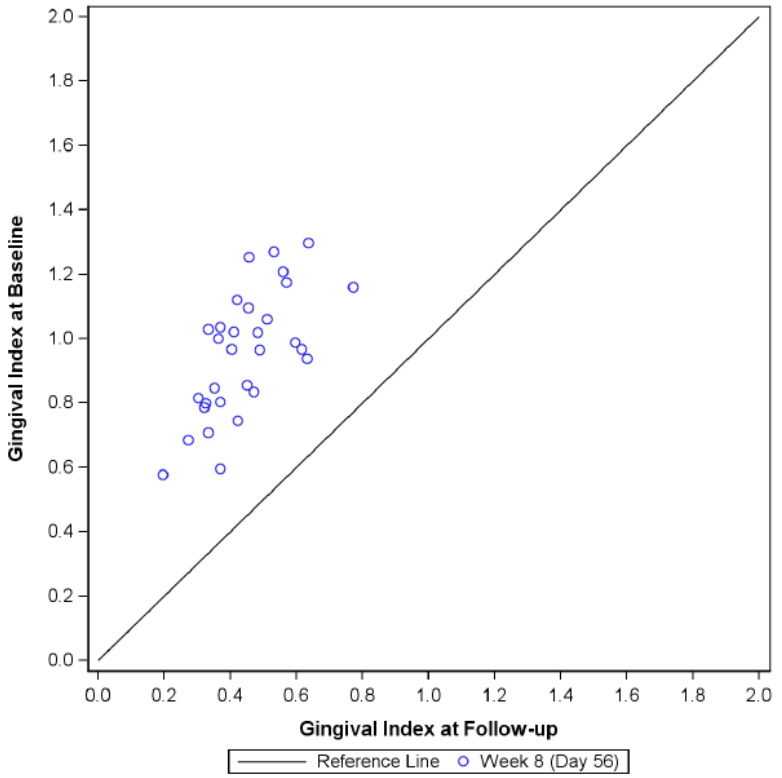


Figure 18. Clinical Trial 1. Reference plot for Gingival Index at week 8 against baseline.
Mean Gingival Index scores plotted above the diagonal line represent subjects that have improved from Baseline to follow-up, these scores represent the combined effects of Intervention 1 and Intervention 2. There are no scores below the line to indicate deterioration in Plaque Index scores from baseline for any subject.

2.6.3 Pocket probing depth (PPD)

Overall mean PPDs were calculated as the arithmetic average of all PPD values per subject. There was little change in mean PPD throughout the study, which would be expected in subjects with gingivitis with no significant loss of attachment. Descriptive statistics are provided for PPD in Table 11. After 4 weeks the reduction in mean PPD was not significantly greater than 0, the mean reduction in PPD with 95% CI was 0.02 (-0.38,0.42). At 8 weeks the reduction in PPDs was not statistically greater than 0, the mean with 95% CI was 0.08 (-0.32,0.48). An analysis of variance indicated no statistically significant difference (p=0.1266).

A line graph showing the reduction in PPD from baseline is shown in Figure 19. Box plots for change from baseline in PPD by visit are presented in Figure 20. Reference plots were produced that provide a visual representation of mean PPD change from baseline at weeks 4 (Figure 21) and 8 weeks (Figure 22).

Visit	n	Mean PPD (SD)	Median PPD	Min, Max	95% CI
Pre-screening	31	1.9 (0.2)	1.9	1.7, 2.6	(1.8, 2.0)
Baseline (Day 0)	31	1.9 (0.2)	1.8	1.6, 2.6	(1.8, 2.0)
Week 4 (Day 28)	31	1.9 (0.2)	1.9	1.6, 2.4	(1.8, 1.9)
Week 8 (Day 56)	31	1.8 (0.2)	1.8	1.4, 2.4	(1.7, 1.9)
Reduction					
Baseline to Week 4	31	0.0 (0.2)	0.0	-0.3, 0.4	(-0.0, 0.1)
Baseline to Week 8	31	0.1 (0.2)	0.1	-0.2, 0.4	(0.0, 0.1)

Table 11. Clinical Trial 1. Descriptive statistics for pocket probing depths including reduction in pocket probing depth from baseline.

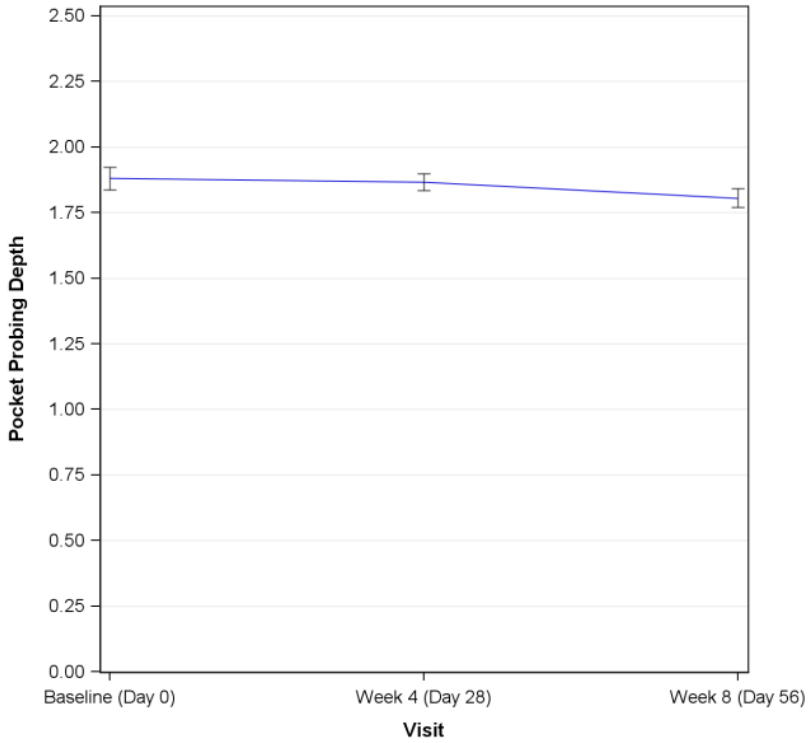


Figure 19. Clinical Trial 1. Line graph to show reduction in pocket probing depth over time with corresponding error bars.
There were no significant reductions in PPDs from baseline for any visit (ANOVA p=0.1266)..

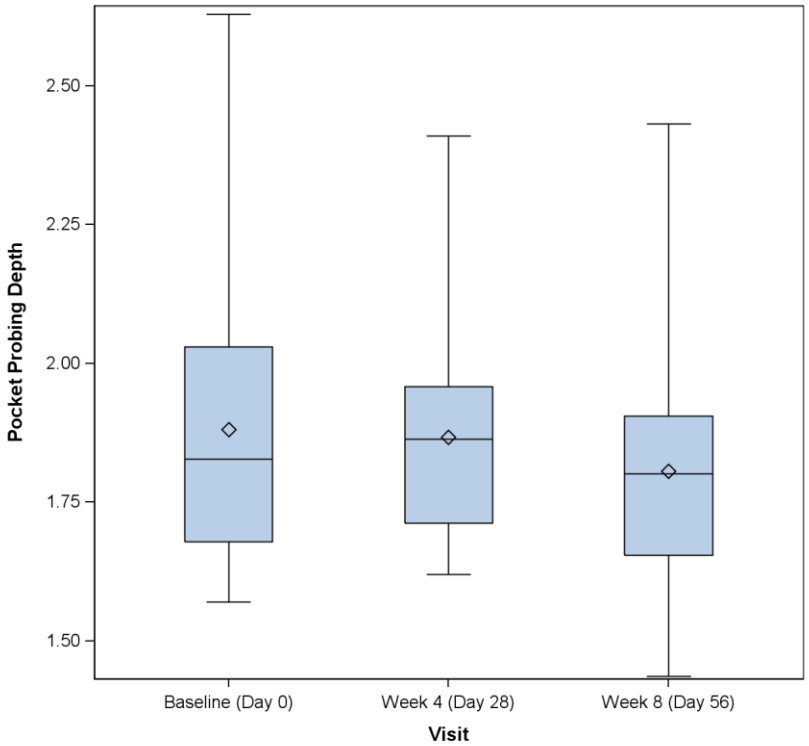


Figure 20. Clinical Trial 1. Box plot for pocket probing depth over time. The interventions took place after the measurements were recorded at baseline (Intervention 1) and week 4 (Intervention 2). Mean values are represented by diamonds and medians by horizontal line though the box plot, error bars are also shown. There were no significant reductions in PPDs from baseline for any visit (ANOVA p=0.1266).

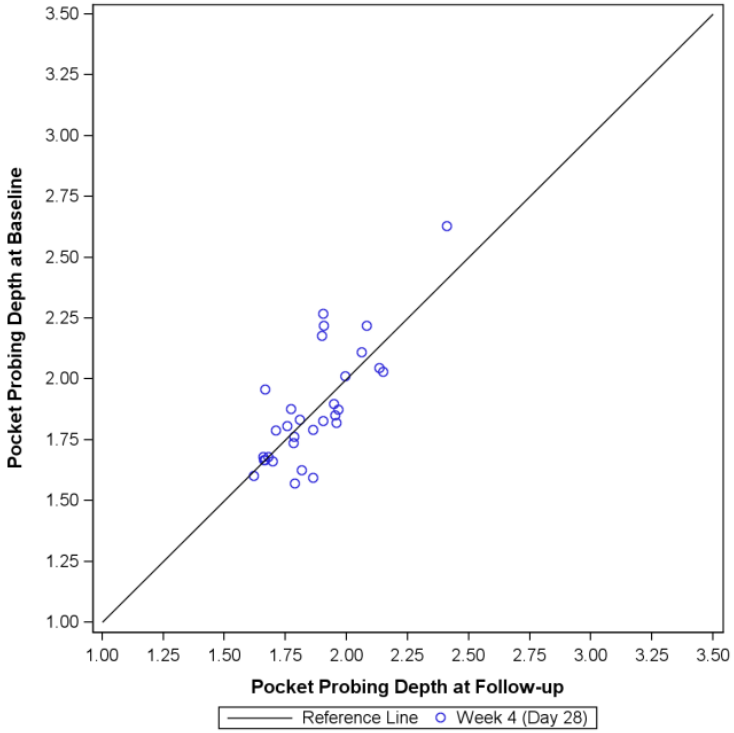


Figure 21. Clinical Trial 1. Reference plot for pocket probing depth at week 4 against baseline.
Mean pocket probing depth scores plotted above the diagonal line represent subjects that have decreased from baseline to week 4, those that fall below the reference line have increased. The plots generally fall either side and close to the reference line indicating little change over time.

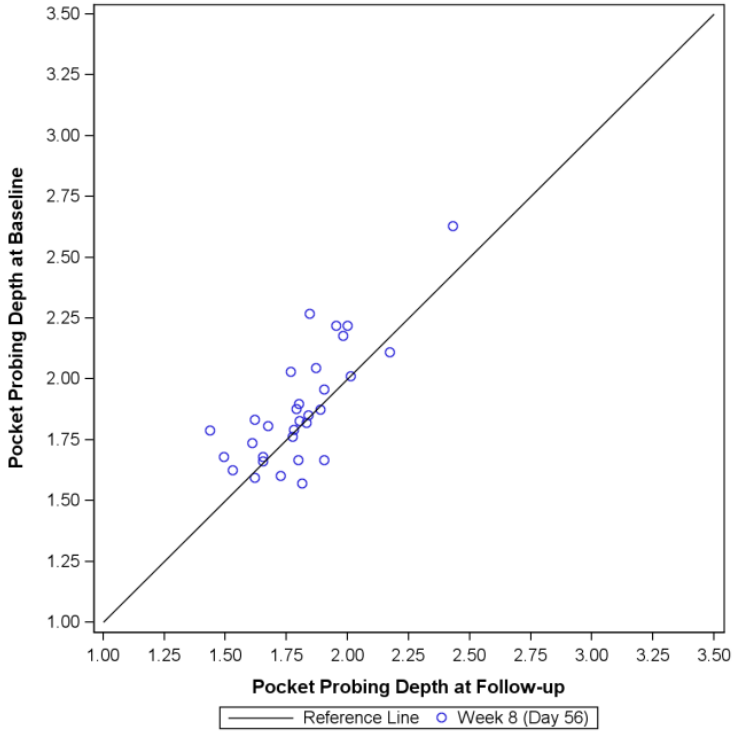


Figure 22. Clinical Trial 1. Reference plot for pocket probing depth at week 8 against baseline.
Mean pocket probing depth scores plotted above the diagonal line represent subjects that have decreased from baseline to week 4, those that fall below the reference line have increased. The plots generally lie close to the reference line indicating little change over time.

2.6.4 Biomarkers

All laboratory analysis was performed blinded to the sample, visit and patient identifiers. Descriptive statistics were produced for each mediator including mean values and standard deviations for each patient at each visit as follows:

- IL-1α (Table 12)
- IL-1β (Table 13)
- IL-8 (Table 14)
- MIP-1α (Table 15)
- MIP-1β (Table 16)
- RANTES (Table 17)
- MMP-1 (Table 18)
- MMP-3 (Table 19)
- MMP-8 (Table 20)
- MMP-9 (Table 21)
- MMP-13 (Table 22)

Log mean values of mediator concentrations in picograms per millilitre were calculated for comparison as the mean values were not normally distributed. Whilst most biomarkers increased transiently post treatment: those that showed statistical significance at the 95% level were IL-1β, MMP-1, MMP-3, MMP-8, MMP-9, from baseline to week 2, RANTES at week 4 and week 8; MIP-1α and MIP-1β at week 8. Figure 23 is a spaghetti plot of the mean log-10 transformed biomarker levels. There were transient changes following the interventions at baseline and week 4 for some mediators. No mediators showed a sustained change from baseline. When examining the collated data or when all biomarkers were considered together, no significant differences were detected over time ($p>0.05$). There were no correlations found with the clinical indices.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min, Max	p-value
IL-1α	Observed	Baseline	31	5.07 (0.64)	5.1	3.74, 6.11	
		Week 2	31	5.16 (0.58)	5.3	3.61, 5.86	
		Week 4	31	5.23 (0.58)	5.3	3.81, 6.29	
		Week 6	30	5.12 (0.66)	5.3	3.84, 6.35	
		Week 8	31	5.22 (0.63)	5.3	3.88, 6.41	
	Reduction from baseline	Week 2	31	-0.09 (0.66)	-0.0	-1.26, 1.44	0.434
		Week 4	31	-0.16 (0.74)	-0.1	-2.20, 1.31	0.236
		Week 6	30	-0.06 (0.78)	-0.1	-1.73, 1.69	0.676
		Week 8	31	-0.15 (0.87)	-0.1	-1.83, 1.41	0.343

Table 12. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for IL-1α over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min, Max	p-value
IL-1β	Observed	Baseline	31	4.57 (0.63)	4.7	2.92, 5.75	
		Week 2	31	4.81 (0.62)	4.9	3.24, 5.94	
		Week 4	31	4.76 (0.66)	4.8	3.00, 6.17	
		Week 6	30	4.81 (0.66)	5.0	3.48, 6.08	
		Week 8	31	4.77 (0.70)	4.8	3.25, 5.87	
	Reduction from baseline	Week 2	31	-0.24 (0.66)	-0.4	-1.53, 1.11	0.050
		Week 4	31	-0.19 (0.74)	-0.1	-2.25, 1.19	0.152
		Week 6	30	-0.25 (0.80)	-0.3	-1.94, 1.66	0.096
		Week 8	31	-0.20 (0.88)	-0.1	-1.83, 1.93	0.211

Table 13. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for IL-1β over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min, Max	p-value
IL-8	Observed	Baseline	31	5.38 (0.52)	5.4	3.96, 6.44	
		Week 2	31	5.60 (0.61)	5.8	4.01, 6.87	
		Week 4	31	5.56 (0.51)	5.6	4.31, 6.28	
		Week 6	30	5.60 (0.64)	5.6	4.32, 6.75	
		Week 8	31	5.58 (0.54)	5.6	4.60, 6.50	
	Reduction from baseline	Week 2	31	-0.22 (0.72)	-0.4	-1.57, 1.49	0.092
		Week 4	31	-0.18 (0.61)	-0.2	-1.45, 1.04	0.115
		Week 6	30	-0.22 (0.89)	-0.2	-2.10, 1.34	0.195
		Week 8	31	-0.20 (0.75)	-0.4	-1.54, 0.95	0.146

Table 14. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for IL-8 over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min, Max	p-value
MIP-1α	Observed	Baseline	31	3.83 (0.44)	3.8	2.93, 4.68	
		Week 2	31	4.03 (0.53)	4.0	3.02, 5.18	
		Week 4	31	4.03 (0.61)	4.0	2.90, 5.54	
		Week 6	30	4.00 (0.46)	3.9	3.28, 4.88	
		Week 8	31	4.07 (0.53)	4.1	3.16, 5.05	
	Reduction from baseline	Week 2	31	-0.20 (0.62)	-0.1	-1.59, 1.03	0.086
		Week 4	31	-0.20 (0.66)	-0.1	-1.81, 1.33	0.109
		Week 6	30	-0.17 (0.66)	-0.1	-1.92, 1.07	0.161
		Week 8	31	-0.24 (0.57)	-0.3	-1.07, 0.82	0.027

Table 15. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for MIP-1α over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min, Max	p-value
MIP-1β	Observed	Baseline	31	3.38 (0.59)	3.4	1.89, 4.64	
		Week 2	31	3.66 (0.74)	3.6	2.42, 5.40	
		Week 4	31	3.58 (0.69)	3.4	2.31, 4.82	
		Week 6	30	3.60 (0.63)	3.3	2.89, 4.83	
		Week 8	31	3.69 (0.68)	3.6	2.56, 5.05	
	Reduction from baseline	Week 2	31	-0.27 (0.90)	-0.3	-2.06, 1.72	0.101
		Week 4	31	-0.19 (0.72)	-0.3	-1.63, 1.20	0.146
		Week 6	30	-0.23 (0.94)	-0.0	-2.41, 1.63	0.196
		Week 8	31	-0.31 (0.74)	-0.4	-1.35 - 1.27	0.028

Table 16. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for MIP-1β over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min, Max	p-value
RANTES	Observed	Baseline	31	3.58 (0.51)	3.6	2.55, 4.44	
		Week 2	31	3.69 (0.42)	3.8	2.84, 4.67	
		Week 4	31	3.92 (0.64)	3.8	2.97, 5.92	
		Week 6	30	3.73 (0.36)	3.7	2.77, 4.65	
		Week 8	31	3.87 (0.45)	3.8	2.98, 4.71	
	Reduction from baseline	Week 2	31	-0.11 (0.55)	-0.1	-0.98, 0.98	0.287
		Week 4	31	-0.34 (0.72)	-0.2	-1.84, 1.16	0.014
		Week 6	30	-0.15 (0.62)	-0.1	-1.65, 1.01	0.186
		Week 8	31	-0.29 (0.61)	-0.3	-1.64, 1.32	0.014

Table 17. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for RANTES over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min, Max	p-value
MMP-1	Observed	Baseline	31	4.56 (0.82)	4.5	2.70, 5.73	
		Week 2	31	4.94 (0.66)	5.0	3.19, 6.24	
		Week 4	31	4.53 (0.62)	4.5	3.13, 6.19	
		Week 6	31	4.83 (0.82)	4.9	3.04, 6.18	
		Week 8	31	4.73 (0.78)	4.9	3.17, 6.26	
	Reduction from baseline	Week 2	31	-0.39 (0.89)	-0.3	-2.90, 1.28	0.021
		Week 4	31	0.03 (1.07)	0.3	-2.90, 1.71	0.886
		Week 6	31	-0.27 (1.04)	-0.2	-2.10, 2.67	0.156
		Week 8	31	-0.17 (1.02)	-0.2	-2.97, 2.01	0.360

Table 18. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for MMP-1 over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min, Max	p-value
MMP-3	Observed	Baseline	31	4.26 (0.74)	4.4	2.47, 5.46	
		Week 2	31	4.60 (0.67)	4.5	2.98, 5.99	
		Week 4	31	4.24 (0.58)	4.3	3.16, 5.29	
		Week 6	31	4.43 (0.73)	4.5	3.11, 6.04	
		Week 8	31	4.30 (0.72)	4.3	2.72, 5.69	
	Reduction from baseline	Week 2	31	-0.34 (0.81)	-0.2	-2.97, 0.76	0.025
		Week 4	31	0.02 (0.81)	0.3	-2.14, 1.16	0.894
		Week 6	31	-0.18 (0.95)	-0.0	-1.81, 2.08	0.312
		Week 8	31	-0.04 (0.90)	0.2	-2.06, 1.59	0.799

Table 19. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for MMP-3 over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min - Max	p-value
MMP-8	Observed	Baseline	31	7.11 (0.76)	7.3	5.77, 8.29	
		Week 2	31	7.68 (0.54)	7.7	6.63, 8.71	
		Week 4	31	7.10 (0.70)	7.3	5.67, 8.74	
		Week 6	31	7.51 (0.81)	7.6	5.16, 8.86	
		Week 8	31	7.16 (0.71)	7.2	5.84, 8.40	
	Reduction from baseline	Week 2	31	-0.57 (0.88)	-0.5	-2.27, 1.26	0.001
		Week 4	31	0.01 (0.94)	0.0	-1.56, 1.78	0.963
		Week 6	31	-0.40 (1.12)	-0.4	-2.40, 2.96	0.057
		Week 8	31	-0.05 (0.96)	-0.1	-1.94, 1.91	0.792

Table 20. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for MMP-8 over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min, Max	p-value
MMP-9	Observed	Baseline	31	7.67 (0.74)	7.7	6.41, 8.96	
		Week 2	31	8.15 (0.54)	8.1	7.10, 9.64	
		Week 4	31	7.68 (0.68)	7.8	6.01, 8.90	
		Week 6	31	7.98 (0.81)	8.1	5.72, 9.27	
		Week 8	31	7.74 (0.79)	7.9	6.13, 9.10	
	Reduction from baseline	Week 2	31	-0.49 (0.88)	-0.3	-2.72, 0.69	0.005
		Week 4	31	-0.02 (0.96)	-0.0	-1.91, 1.99	0.921
		Week 6	31	-0.32 (1.18)	-0.2	-2.35, 3.24	0.145
		Week 8	31	-0.08 (0.95)	-0.2	-2.18, 1.61	0.651

Table 21. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for MMP-9 over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

Biomarker	Endpoint	Visit	n	Mean (SD)	Median	Min, Max	p-value
MMP-13	Observed	Baseline	31	5.23 (0.86)	5.3	3.01, 6.41	
		Week 2	31	5.39 (0.90)	5.6	3.44, 7.16	
		Week 4	31	5.26 (0.82)	5.4	2.74, 6.79	
		Week 6	31	5.41 (0.91)	5.8	3.44, 6.50	
		Week 8	31	5.44 (0.95)	5.5	3.45, 7.09	
	Reduction from baseline	Week 2	31	-0.16 (0.84)	-0.1	-1.65, 1.42	0.296
		Week 4	31	-0.03 (0.97)	0.1	-2.15, 1.82	0.851
		Week 6	31	-0.18 (1.02)	-0.1	-1.67, 2.65	0.329
		Week 8	31	-0.21 (1.03)	-0.2	-2.46, 1.91	0.264

Table 22. Clinical Trial 1. Mean observed log-10 transformed biomarker levels for MMP-13 over the 8 weeks of the study.
The lower portion of the table indicates reduction from baseline with negative values for mean reduction indicating a positive change in biomarkers, all biomarker concentrations are in pg/ml.

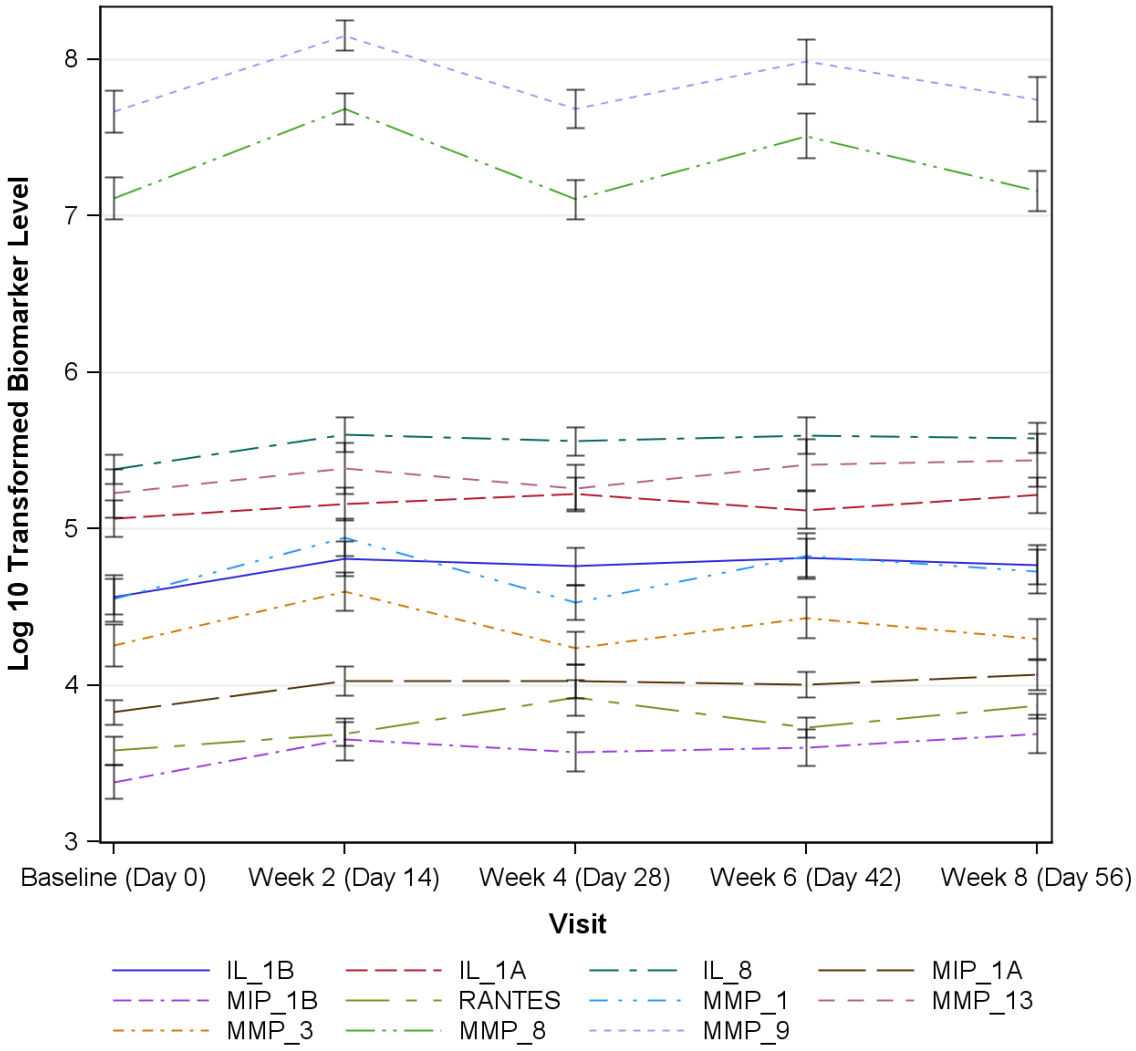


Figure 23. Clinical Trial 1. A spaghetti plot representing the mean log 10 transformed biomarker levels by visit.
Vertical bars show standard error of the mean, all biomarker concentrations are in pg/ml.

2.6.5 Microbial biomarkers

All laboratory analysis was performed blinded to the sample, visit and patient identifers. Following examination of the distribution of the microbial data it was log 10 transformed prior to analysis. Descriptive statistics for mean microbial levels are presented along with t-test p-values showing change from baseline (Table 23 for subgingival species and Table 24 for supragingival species). Summary tables of those species with significant reductions from baseline are shown in Table 25 for the subgingival samples and Table 26 for the supragingival samples. Those species with significant increases from baseline are shown in Table 27 for subgingival samples and Table 28 for the supragingival samples.

Several organisms associated with disease significantly decreased post intervention (Table 25 and Table 26). Some of the bacteria more commonly associated with oral health: *Streptococcus mitis*, *Streptococcus sanguinis*, *Veillonella parvula* increased significantly post treatment. Three species that are associated with disease: *Granulicatella adjacens*, *Neisseria mucosa*, and *Prevotella nigrescens* increased significantly post treatment. Species associated with deep pocketing, bleeding upon probing and periodontitis: *Porphyromonas gingivalis*, *Tannerella forsynthesis*, and *Treponema denticola* (Figure 24) were found in small numbers in all subjects with little change over time (Socransky *et al.*, 1998).

Table 23. Clinical Trial 1. Descriptive summary of subgingival bacterial species including ANOVA statistical difference from baseline (log 10 transformed data).

Microbe	Visit	n	Mean (SD)	Median	Min, Max	p-value change from baseline
Actinomyces naeslundii	Baseline	27	0.14 (0.27)	0.0	0.00, 0.88	N/a
	Week 4	27	0.23 (0.33)	0.0	0.00, 0.99	0.151
	Week 8	27	0.08 (0.30)	0.0	-0.47,0.95	0.263
Actinomyces odontolyticus	Baseline	27	0.02 (0.07)	0.0	0.00, 0.27	N/a
	Week 4	27	0.04 (0.12)	0.0	0.00, 0.52	0.381
	Week 8	27	-0.03 (0.28)	0.0	-0.91, 0.64	0.375
Actinomyces viscosus	Baseline	27	0.09 (0.21)	0.0	0.00, 0.71	N/a
	Week 4	27	0.02 (0.07)	0.0	0.00, 0.26	0.128
	Week 8	27	-0.04 (0.16)	0.0	-0.71, 0.00	0.010
Aggregatibacter actinomycetemcomitans	Baseline	27	-0.01 (0.06)	0.0	-0.28, 0.11	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.597
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	0.597
Bifidobacterium infantis	Baseline	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
Campylobacter gracilis	Baseline	27	0.46 (0.37)	0.5	0.00, 1.09	N/a
	Week 4	27	0.13 (0.24)	0.0	0.00, 0.79	<0.001
	Week 8	27	0.06 (0.23)	0.0	-0.47, 0.54	<0.001
Capnocytophaga ochracea	Baseline	27	0.24 (0.31)	0.2	0.00, 1.15	N/a
	Week 4	27	0.10 (0.18)	0.0	0.00, 0.56	0.062
	Week 8	27	-0.07 (0.26)	0.0	-0.92, 0.37	<0.001
Catonella morbi	Baseline	27	0.03 (0.32)	0.0	-0.63, 0.81	N/a
	Week 4	27	0.05 (0.13)	0.0	0.00, 0.60	0.765
	Week 8	27	0.04 (0.09)	0.0	0.00, 0.27	0.875
Dialister pneumosintes	Baseline	27	0.87 (0.47)	0.9	0.00, 1.56	N/a
	Week 4	27	0.23 (0.37)	0.0	0.00, 1.09	<0.001
	Week 8	27	0.27 (0.30)	0.2	0.00, 0.88	<0.001
Enterococcus faecalis	Baseline	27	-0.00 (0.21)	0.0	-0.88, 0.58	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.980
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	0.980
Eubacterium brachy	Baseline	27	0.10 (0.23)	0.0	0.00, 0.93	N/a
	Week 4	27	0.02 (0.06)	0.0	0.00, 0.23	0.073
	Week 8	27	0.07 (0.15)	0.0	0.00, 0.63	0.543
Eubacterium saburreum	Baseline	27	0.30 (0.34)	0.2	0.00, 1.09	N/a
	Week 4	27	0.12 (0.24)	0.0	0.00, 0.75	0.026
	Week 8	27	0.10 (0.23)	0.0	-0.11, 0.79	0.014

Table 23. (Continued)

Fillifactor alocis	Baseline	27	-0.02 (0.12)	0.0	-0.60, 0.00	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.282
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	0.282
Fusobacterium nucleatum	Baseline	27	-0.04 (0.22)	0.0	-0.96, 0.23	N/a
	Week 4	27	-0.03 (0.14)	0.0	-0.64, 0.08	0.957
	Week 8	27	-0.02 (0.18)	0.0	-0.49, 0.26	0.779
Granulicatella adjacens	Baseline	27	0.04 (0.22)	0.0	-0.64, 0.38	N/a
	Week 4	27	0.23 (0.28)	0.0	0.00, 0.92	<0.001
	Week 8	27	0.22 (0.28)	0.1	-0.19, 0.83	<0.001
Lactobacillus gasseri	Baseline	27	-0.02 (0.10)	0.0	-0.49, 0.00	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.327
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	0.327
Lactobacillus reuteri	Baseline	27	-0.00 (0.00)	0.0	-0.01, 0.00	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.327
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	0.327
Leptotrichia buccalis	Baseline	27	-0.04 (0.17)	0.0	-0.62, 0.30	N/a
	Week 4	27	0.06 (0.24)	0.0	0.00, 1.13	0.085
	Week 8	27	0.07 (0.19)	0.0	0.00, 0.78	0.057
Neisseria mucosa	Baseline	27	0.02 (0.07)	0.0	0.00, 0.28	N/a
	Week 4	27	0.17 (0.30)	0.0	0.00, 1.02	0.020
	Week 8	27	0.18 (0.35)	0.0	-0.80, 0.83	0.020
Parvimonas micra	Baseline	27	0.21 (0.37)	0.0	0.00, 1.11	N/a
	Week 4	27	0.02 (0.08)	0.0	-0.11, 0.24	0.016
	Week 8	27	0.02 (0.07)	0.0	-0.01, 0.25	0.009
Porphyromonas gingivalis	Baseline	27	0.01 (0.05)	0.0	0.00, 0.26	N/a
	Week 4	27	-0.07 (0.27)	0.0	-1.07, 0.00	0.154
	Week 8	27	-0.07 (0.28)	0.0	-1.14, 0.10	0.132
Prevotella nigrescens	Baseline	27	-0.12 (0.29)	0.0	-0.88, 0.28	N/a
	Week 4	27	0.01 (0.06)	0.0	0.00, 0.29	0.031
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	0.046
Prevotella oris	Baseline	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 8	27	-0.03 (0.16)	0.0	-0.84, 0.00	0.327
Propionibacterium propionicus	Baseline	27	0.02 (0.10)	0.0	0.00, 0.52	N/a
	Week 4	27	0.03 (0.08)	0.0	0.00, 0.30	0.7909
	Week 8	27	0.03 (0.24)	0.0	-0.65, 0.58	0.911
Pseudomonas aeruginosa	Baseline	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
Selenomonas noxia	Baseline	27	0.41 (0.42)	0.5	0.00, 1.28	N/a
	Week 4	27	0.06 (0.15)	0.0	0.00, 0.61	<0.001
	Week 8	27	0.00 (0.11)	0.0	-0.43, 0.30	<0.001

Table 23. (Continued)

Selenomonas sputigena	Baseline	27	0.19 (0.33)	0.0	0.00, 1.00	N/a
	Week 4	27	0.06 (0.13)	0.0	0.00, 0.55	0.059
	Week 8	27	0.07 (0.16)	0.0	-0.01, 0.56	0.107
Streptococcus mitis	Baseline	27	0.17 (0.29)	0.0	0.00, 1.10	N/a
	Week 4	27	0.47 (0.54)	0.0	0.00, 1.38	0.027
	Week 8	27	0.59 (0.49)	0.6	-0.35, 1.28	<0.001
Streptococcus mutans	Baseline	27	-0.12 (0.42)	0.0	-2.08, 0.00	N/a
	Week 4	27	0.04 (0.12)	0.0	0.00, 0.54	0.066
	Week 8	27	-0.07 (0.21)	0.0	-0.91, 0.00	0.594
Streptococcus sanguinis	Baseline	27	0.59 (0.42)	0.6	0.00, 1.38	N/a
	Week 4	27	0.90 (0.37)	1.0	0.00, 1.43	0.002
	Week 8	27	0.93 (0.40)	1.0	-0.70, 1.40	<0.001
Tannerella forsynthesis	Baseline	27	0.03 (0.18)	0.0	0.00, 0.94	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.327
	Week 8	27	-0.01 (0.04)	0.0	-0.23, 0.00	0.233
Treponema denticola	Baseline	27	0.35 (0.35)	0.3	0.00, 1.05	N/a
	Week 4	27	0.10 (0.22)	0.0	0.00, 0.71	<0.001
	Week 8	27	-0.04 (0.29)	0.0	-0.66, 0.73	<0.001
Veillonella parvula	Baseline	27	1.00 (0.34)	1.0	0.24, 1.58	N/a
	Week 4	27	1.31 (0.23)	1.3	0.52, 1.66	<0.001
	Week 8	27	1.35 (0.17)	1.4	1.09, 1.65	<0.001

Table 24. Clinical Trial 1. Descriptive summary of supragingival bacterial species including ANOVA statistical difference from baseline (log 10 transformed data).

Microbe	Visit	n	Mean (SD)	Median	Min, Max	p-value change from baseline
Actinomyces naeslundii	Baseline	27	0.40 (0.41)	0.3	0.00, 1.43	N/a
	Week 4	27	0.25 (0.27)	0.2	0.00, 0.83	0.164
	Week 8	27	0.26 (0.33)	0.1	0.00, 1.16	0.223
Actinomyces odontolyticus	Baseline	27	0.04 (0.17)	0.0	0.00, 0.84	N/a
	Week 4	27	0.01 (0.04)	0.0	0.00, 0.22	0.359
	Week 8	27	-0.08 (0.25)	0.0	-0.94, 0.00	0.041
Actinomyces viscosus	Baseline	27	-0.01 (0.23)	0.0	-0.63, 0.55	N/a
	Week 4	27	0.03 (0.15)	0.0	-0.43, 0.54	0.483
	Week 8	27	0.06 (0.18)	0.0	-0.01, 0.71	0.184
Aggregatibacter actinomycetemcomitans	Baseline	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
Bifidobacterium infantis	Baseline	27	0.02 (0.11)	0.0	0.00, 0.55	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.327
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	0.327
Campylobacter gracilis	Baseline	27	0.25 (0.28)	0.2	0.00, 0.81	N/a
	Week 4	27	0.08 (0.20)	0.0	0.00, 0.84	0.017
	Week 8	27	0.04 (0.23)	0.0	-0.94, 0.34	0.004
Capnocytophaga ochracea	Baseline	27	0.33 (0.44)	0.2	0.00, 1.49	N/a
	Week 4	27	0.13 (0.20)	0.0	0.00, 0.83	0.039
	Week 8	27	0.21 (0.31)	0.0	-0.03,1.15	0.274
Catonella morbi	Baseline	27	0.01 (0.06)	0.0	0.00, 0.34	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.327
	Week 8	27	-0.03 (0.18)	0.0	-0.92,0.00	0.205
Dialister pneumosintes	Baseline	27	-0.08 (0.26)	0.0	-0.93, 0.18	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.132
	Week 8	27	0.02 (0.07)	0.0	0.00, 0.29	0.066
Enterococcus faecalis	Baseline	27	-0.05 (0.33)	0.0	-1.70, 0.24	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.404
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	0.404
Eubacterium brachy	Baseline	27	0.21 (0.29)	0.0	0.00, 0.94	N/a
	Week 4	27	0.15 (0.26)	0.0	0.00, 0.85	0.487
	Week 8	27	0.03 (0.15)	0.0	-0.45, 0.27	0.014
Eubacterium saburreum	Baseline	27	0.06 (0.14)	0.0	0.00, 0.55	N/a
	Week 4	27	0.04 (0.12)	0.0	0.00, 0.54	0.607
	Week 8	27	-0.08 (0.33)	0.0	-0.92, 0.83	0.054

Table 24. (Continued)

Fillifactor alocis	Baseline	27	-0.06 (0.26)	0.0	-0.93, 0.34	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.271
	Week 8	27	0.01 (0.05)	0.0	0.00, 0.24	0.208
Fusobacterium nucleatum	Baseline	27	0.05 (0.15)	0.0	0.00, 0.71	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.119
	Week 8	27	-0.03 (0.14)	0.0	-0.63, 0.25	0.126
Granulicatella adjacens	Baseline	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 4	27	0.06 (0.16)	0.0	0.00, 0.72	0.075
	Week 8	27	0.04 (0.14)	0.0	0.00, 0.74	0.185
Lactobacillus gasseri	Baseline	27	0.16 (0.26)	0.0	0.00, 0.84	N/a
	Week 4	27	0.08 (0.15)	0.0	0.00, 0.62	0.052
	Week 8	27	-0.01 (0.21)	0.0	-0.63, 0.34	0.009
Lactobacillus reuteri	Baseline	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
Leptotrichia buccalis	Baseline	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 8	27	0.01 (0.05)	0.0	0.00, 0.27	0.327
Neisseria mucosa	Baseline	27	0.03 (0.14)	0.0	0.00, 0.71	N/a
	Week 4	27	0.10 (0.22)	0.0	0.00, 0.82	0.026
	Week 8	27	0.01 (0.30)	0.0	-0.62, 0.83	0.774
Parvimonas micra	Baseline	27	0.04 (0.19)	0.0	0.00, 0.97	N/a
	Week 4	27	0.02 (0.06)	0.0	0.00, 0.24	0.495
	Week 8	27	0.03 (0.08)	0.0	0.00, 0.26	0.664
Porphyromonas gingivalis	Baseline	27	0.03 (0.11)	0.0	0.00, 0.53	N/a
	Week 4	27	0.03 (0.09)	0.0	0.00, 0.34	0.963
	Week 8	27	0.01 (0.05)	0.0	0.00, 0.27	0.440
Prevotella nigrescens	Baseline	27	0.09 (0.21)	0.0	0.00, 0.89	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	0.037
	Week 8	27	0.02 (0.07)	0.0	0.00, 0.26	0.044
Prevotella oris	Baseline	27	-0.03 (0.18)	0.0	-0.92, 0.00	N/a
	Week 4	27	0.01 (0.05)	0.0	0.00, 0.24	0.230
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	0.327
Propionibacterium propionicus	Baseline	27	0.05 (0.33)	0.0	-0.63, 0.95	N/a
	Week 4	27	0.02 (0.06)	0.0	0.00, 0.24	0.581
	Week 8	27	0.02 (0.06)	0.0	0.00, 0.24	0.625
Pseudomonas aeruginosa	Baseline	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 4	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	N/a
Selenomonas noxia	Baseline	27	0.20 (0.43)	0.0	-0.63, 1.24	N/a
	Week 4	27	0.02 (0.10)	0.0	0.00, 0.54	0.041
	Week 8	27	0.04 (0.09)	0.0	0.00, 0.27	0.056

Table 24. (Continued)

Selenomonas sputigena	Baseline	27	0.74 (0.57)	0.8	0.00, 1.56	N/a
	Week 4	27	0.31 (0.39)	0.2	0.00, 1.24	<0.001
	Week 8	27	0.25 (0.31)	0.0	0.00, 0.95	<0.001
Streptococcus mitis	Baseline	27	0.44 (0.40)	0.5	0.00, 1.26	N/a
	Week 4	27	0.65 (0.51)	0.7	0.00, 1.56	0.013
	Week 8	27	0.63 (0.54)	0.7	0.00, 1.46	0.035
Streptococcus mutans	Baseline	27	0.02 (0.07)	0.0	0.00, 0.27	N/a
	Week 4	27	0.03 (0.11)	0.0	0.00, 0.54	0.702
	Week 8	27	-0.08 (0.24)	0.0	-0.92, 0.00	0.041
Streptococcus sanguinis	Baseline	27	0.56 (0.41)	0.6	0.00, 1.11	N/a
	Week 4	27	0.95 (0.45)	1.1	0.00, 1.47	<0.001
	Week 8	27	0.96 (0.43)	1.1	0.00, 1.52	<0.001
Tannerella forsynthesis	Baseline	27	0.01 (0.05)	0.0	0.00, 0.24	N/a
	Week 4	27	0.01 (0.05)	0.0	0.00, 0.24	1.000
	Week 8	27	-0.07 (0.25)	0.0	-0.93, 0.00	0.118
Treponema denticola	Baseline	27	-0.03 (0.29)	0.0	-0.94, 0.34	N/a
	Week 4	27	0.01 (0.05)	0.0	0.00, 0.24	0.480
	Week 8	27	0.00 (0.00)	0.0	0.00, 0.00	0.577
Veillonella parvula	Baseline	27	0.59 (0.34)	0.6	0.00, 1.31	N/a
	Week 4	27	0.81 (0.37)	0.8	0.00, 1.48	0.028
	Week 8	27	0.79 (0.35)	0.8	0.00, 1.30	0.038

Microbe	Visit	n	Mean (SD)	Median	Min, Max	p-value change from baseline
Dialister pneumonistes	Week 4	27	0.63 (0.58)	0.8	-1.09, 1.55	<0.001
	Week 8	27	0.59 (0.61)	0.7	-0.57, 1.56	<0.001
Campylobacter gracilis	Week 4	27	0.34 (0.41)	0.2	-0.22, 1.09	<0.001
	Week 8	27	0.41 (0.44)	0.3	-0.37, 1.26	<0.001
Selenomonas noxia	Week 4	27	0.35 (0.42)	0.1	-0.26, 1.22	<0.001
	Week 8	27	0.41 (0.46)	0.5	-0.30, 1.28	<0.001
Treponema denticola	Week 4	27	0.25 (0.35)	0.2	-0.56, 1.05	<0.001
	Week 8	27	0.39 (0.48)	0.3	0.00, 1.61	<0.001
Capnocytophaga ochracea	Week 4	27	0.14 (0.38)	0.0	-0.56, 1.15	0.062
	Week 8	27	0.31 (0.41)	0.3	-0.37, 1.58	<0.001
Eubacterium saburreum	Week 4	27	0.18 (0.39)	0.0	-0.61, 0.90	0.026
	Week 8	27	0.20 (0.39)	0.0	-0.59, 0.94	0.014
Parvimonas micra	Week 4	27	0.19 (0.37)	0.0	-0.24, 1.11	0.016
	Week 8	27	0.19 (0.34)	0.0	0.00, 1.11	0.009
Actinomyces viscosus	Week 4	27	0.07 (0.22)	0.0	-0.26, 0.71	0.128
	Week 8	27	0.13 (0.24)	0.0	0.00, 0.71	0.010

Table 25. Clinical Trial 1. Subgingival species with statistically significant reductions at respective weeks (log 10 transformed data).

Microbe	Visit	n	Mean (SD)	Median	Min, Max	p-value change from baseline
Selenomonas sputigena	Week 4	27	0.44 (0.59)	0.5	-0.88 ,1.38	<0.001
	Week 8	27	0.50 (0.63)	0.5	-0.74, 1.56	<0.001
Campylobacter gracilis	Week 4	27	0.17 (0.36)	0.0	-0.84, 0.81	0.017
	Week 8	27	0.21 (0.36)	0.0	-0.26, 0.94	0.004
Capnocytophaga ochracea	Week 4	27	0.20 (0.47)	0.0	-0.83, 1.15	0.039
	Week 8	27	0.12 (0.55)	0.0	-1.15, 1.49	0.274
Eubacterium brachy	Week 4	27	0.06 (0.44)	0.0	-0.85, 0.94	0.487
	Week 8	27	0.18 (0.36)	0.0	-0.27, 0.94	0.014
Selenomonas noxia	Week 4	27	0.18 (0.42)	0.0	-0.63, 1.24	0.041
	Week 8	27	0.16 (0.41)	0.0	-0.63, 1.00	0.056
Lactobacillus gasseri	Week 4	27	0.09 (0.22)	0.0	-0.24, 0.68	0.052
	Week 8	27	0.17 (0.31)	0.0	-0.34, 0.63	0.009
Actinomyces odontolyticus	Week 4	27	0.03 (0.17)	0.0	-0.22, 0.84	0.359
	Week 8	27	0.12 (0.29)	0.0	0.00, 0.94	0.041
Streptococcus mutans	Week 8	27	0.10 (0.24)	0.0	0.00, 0.92	0.041
Prevotella nigrescens	Week 4	27	0.09 (0.21)	0.0	0.00, 0.89	0.037
	Week 8	27	0.07 (0.17)	0.0	-0.02, 0.63	0.044
Neisseria mucosa	Week 8	27	0.02 (0.32)	0.0	-0.83, 0.71	0.774

Table 26. Clinical Trial 1. Supragingival species with statistically significant reductions at respective weeks (log 10 transformed data).

Microbe	Visit	n	Mean (SD)	Median	Min, Max	p-value change from baseline
Streptococcus mitis	Week 4	27	-0.30 (0.67)	0.0	-1.38, 1.10	0.027
	Week 8	27	-0.42 (0.50)	-0.2	-1.28, 0.35	<0.001
Veillonella parvula	Week 4	27	-0.31 (0.36)	-0.3	-1.21, 0.56	<0.001
	Week 8	27	-0.35 (0.32)	-0.3	-1.30, 0.04	<0.001
Streptococcus sanguinis	Week 4	27	-0.31 (0.46)	-0.2	-1.29, 0.52	0.002
	Week 8	27	-0.34 (0.46)	-0.2	-1.16, 0.70	<0.001
Granulicatella adjacens	Week 4	27	-0.19 (0.23)	-0.1	-0.68, 0.11	<0.001
	Week 8	27	-0.19 (0.24)	-0.2	-0.64, 0.30	<0.001
Neisseria mucosa	Week 4	27	-0.15 (0.31)	0.0	-1.02, 0.28	0.020
	Week 8	27	-0.16 (0.33)	0.0	-0.83, 0.80	0.020
Prevotella nigrescens	Week 4	27	-0.13 (0.29)	0.0	-0.88, 0.28	0.031
	Week 8	27	-0.12 (0.29)	0.0	-0.88, 0.28	0.046

Table 27. Clinical Trial 1. Subgingival species with statistically significant increases at respective weeks (log 10 transformed data).

Microbe	Visit	n	Mean (SD)	Median	Min, Max	p-value change from baseline
Streptococcus sanguinis	Week 4	27	-0.39 (0.47)	-0.4	-1.19, 0.88	<0.001
	Week 8	27	-0.40 (0.46)	-0.4	-1.22, 0.88	<0.001
Veillonella parvula	Week 4	27	-0.23 (0.51)	-0.3	-1.09, 1.09	0.028
	Week 8	27	-0.20 (0.47)	-0.1	-1.00, 0.54	0.038
Streptococcus mitis	Week 4	27	-0.21 (0.40)	-0.2	-0.70, 0.85	0.013
	Week 8	27	-0.19 (0.45)	-0.2	-0.84, 0.85	0.035
Streptococcus mutans	Week 4	27	-0.01 (0.13)	0.0	-0.54, 0.27	0.702
Neisseria mucosa	Week 4	27	-0.07 (0.16)	0.0	-0.68, 0.00	0.026

Table 28. Clinical Trial 1. Supragingival species with statistically significant increases at respective weeks (log 10 transformed data).

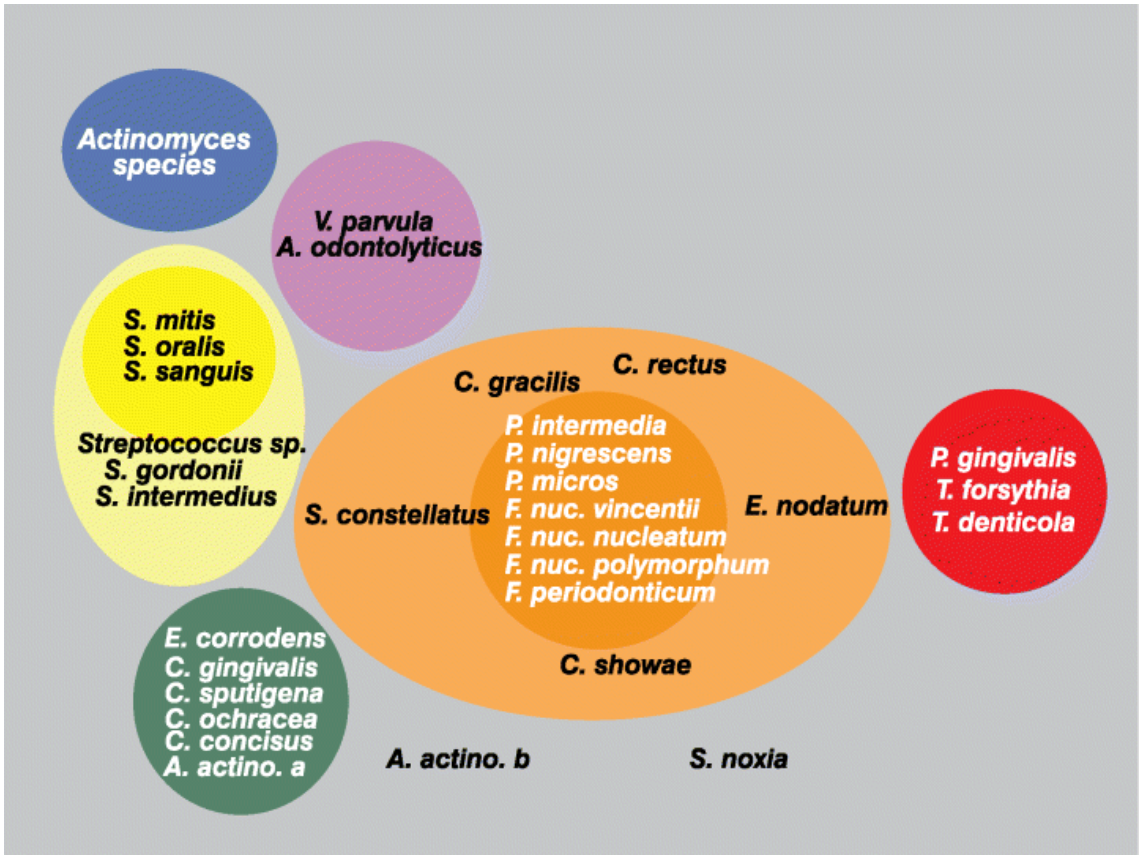


Figure 24. Diagrammatic representation of the relationships within and between microbial complexes (Socransky et al., 1998).
The species to the left of the schematic being representative of commensal species and those associated with health moving to the right of the diagram representing those organisms that were particularly associated with progressive periodontal attachment loss.

2.7 Discussion

The changes in microbial populations following hygiene phase treatment either brushing alone or with adjunctive professional prophylaxis are consistent with previous studies (Aspiras et al., 2008; Barros et al., 2008; Offenbacher et al., 2010; Salvi et al., 2010). These indicate a progressive increase in the proportion of bacteria associated with disease to those associated with health in both supragingival and subgingival plaque (Kumar et al., 2006). These microbial shifts generally precede changes in clinical manifestations that are indicative of disease development or resolution. In this study, this is apparent post intervention with significant increases observed with 'health-associated' bacteria (*Streptococcus mitis, Streptococcus sanguinis* and *Veillonella parvula*) and significant decreases in disease associated bacteria. Reductions in levels are observed for *Dialister pneumosintes* and *Selenomonas noxia* associated with gingivitis as well as for *Treponema denticola* of the 'red complex' of subgingival bacteria. The latter reside in the subgingival periodontal pocket and are typically associated with advanced periodontal disease.

As with many microbial ecosystems, bacterial interactions occur both chemically and physically. These interactions in turn drive cellular spatial arrangements within bacterial communities in plaque biofilm. Mechanically disrupting the biofilm by tooth brushing or professional prophylaxis disrupts the arrangements of a commensal or pathogenic bacterial biofilm. The effect of these interventions results in the creation of a favourable environment for non-pathogenic bacteria. Removal of the upper layers of subgingival plaque and associated endotoxins will 'expose' the predominantly pathogenic anaerobic species embedded deeper in the biofilm; potentially exposing subgingival species to a more hostile environment of high redox potential (Smulow et al., 1983; Katsanoulas et al., 1992). This favours a change in the composition of plaque in favour of those species associated with health that are aerobic and enhance the antagonistic relationship that exists between pathogenic and non-pathogenic species (Smulow et al., 1983; Dahlen et al., 1992; Socransky et al., 1998). The effect of compliance with an oral hygiene regimen and supplemented by professional prophylaxis may account for the increased levels of beneficial bacteria, decrease in disease associated species, and concomitant clinical observations of plaque reduction and improved gingival

health. (Haffajee et al., 2001a; Haffajee et al., 2001b). The findings of this study are in keeping with previous evidence that optimal supragingival plaque control affects the quantity of plaque biofilm and also its composition (Teles et al., 2006). Techniques using DNA probes for microbiological analysis allow the potential for a vast number of bacterial species to be identified; with this ever-increasing number of species identifying those species that are predictive of progressive disease may become more difficult, and care must be taken when drawing conclusions about the pathogenicity of specific species. The aim of treatment of gingivitis should be to reduce the quantity of bacterial pathogens to a level that is tolerated by the host and can be maintained by self-performed plaque control and more infrequent professional intervention (Teles et al., 2006). There is evidence to suggest that the risk of developing periodontitis increases when red complex bacteria are present at greater numbers at deeper sites with clinical attachment loss (Haffajee and Socransky, 1994; Teles et al., 2006). There is, however, no measurable bacterial threshold over which gingivitis will progress to periodontitis, the response of each individual to pathogenic organisms will vary and cause-related therapy should continue to be initiated to reduce the numbers of key pathogenic species (Chapple, 2009).

Inflammation occurs in gingival tissue in response to adjacent plaque bacterial biofilm and the accumulation of endotoxins. At the tissue and cellular level, clinical changes in gingivitis are caused by an initial increase in blood flow and enhanced vascular permeability. This results from the influx of cells (neutrophils and macrophages) from the peripheral blood to the gingival crevice. The cytokines (IL-1α, IL-1β and IL-8) and chemokines (MIP-1α, MIP-1β, RANTES) investigated in this study play a major role in activating and recruiting these inflammatory cells to the site of infection (Offenbacher et al., 2010). The MMPs are molecules (enzymes) involved in the destruction of periodontal tissue and are often associated with more advanced stages of periodontal disease and overt clinical signs (Birkedal-Hansen, 1993). Bleeding and inflammation resulting from these events form the basis of the gingival indices currently used to monitor and diagnose gingivitis.

During hygiene phase therapy for treatment of clinical signs of inflammation, the a priori hypothesis would be that reductions in clinical indices, particularly GI would be concomitant with reductions in subclinical levels of cytokine, chemokine and

MMP biomarkers. In this study there were clinical improvements following each intervention but no significant reductions in GCF inflammatory mediators. The absence of statistically significant differences in levels of specific inflammatory biomarkers may be due to high biological variance in subjects' genetic disposition (better or poorer responders to treatment than others), microbial flora in inflamed sites and the magnitude of the host inflammatory response, all of which reduce the power to detect treatment effects.

The bacterial content of the pocket is an important factor in gene expression in the gingival tissues and therefore, can influence the clinical phenotype and inflammatory response. Pathogenic bacteria may directly up-regulate only a few genes responsible for a generalised non-specific inflammatory response while regulating a larger set of genes responsible for localised cellular responses unique to each bacterium in the biofilm. The variation in the microbial-host gene regulation could explain why a small change in biomarker activity for one individual may not translate to an equivalent degree of clinical change in another. It also reinforces the challenges of ascribing predictable trends of some biomarker behaviours to specific periodontal disease states or indeed generally to inflammatory disease.

In this study there was a general, although statistically insignificant, trend for a number of biomarkers to increase at week 2 (following the first intervention), decrease at week 4 and then a repeat in this trend at weeks 6 and 8 (following the second intervention). There were some reductions of individual IL cytokines and chemokine biomarkers from baseline, but these were not sustained reductions throughout the study. A previous study by Honda et al. found that pro-inflammatory cytokines such as IL-1β and TNF- α (not investigated in this study) may play a role in gingivitis that may differ from the role they play in more advanced periodontitis (Honda et al., 2006). IL-1β is known to up-regulate tissue turnover, it may not necessarily induce tissue destruction in gingivitis lesions, but may stimulate MMPs at the same time (Lark et al., 1990). Inflammatory biomarkers are not necessarily always pathogenic and exhibit different functions; MMPs being involved in tissue remodelling however in excess can exacerbate inflammation driven tissue damage.

It is possible that the type and severity of the host response is dependent upon the stage of the periodontal lesion. In this study, subjects with a diagnosis of mild to moderate gingivitis were recruited. It is possible that a transient increase in IL-1β and other cytokine levels at the 2 week sampling time point may have been sufficient to stimulate collagen synthesis for tissue repair; any further increase may have been counterproductive to the host and levels then decrease. The same biomarker might behave differently in advanced periodontal disease perhaps by being unregulated, ultimately leading to irreversible tissue damage. It is also possible that other biomarkers play contradictory roles in the inflammatory cascade, where they may help initially with normal tissue turnover (or regulation of strictly inflammatory biomarkers) in the early stages of inflammation they then may acquire a pathogenic role if unregulated. This transient beneficial role may explain why some of these biomarkers appear to increase at the 2-week time point while clinically gingival health continues to improve.

Implementation of a sustained oral regimen and/or professional cleaning may have helped prevent continued accumulation of some of these biomarkers to pathogenic inflammatory levels. Inflammatory mediators and biofilm accumulating in the gingival crevice was removed at regular intervals. This could account for why most of the biomarker improvements were seen at the maximum time point (e.g. 4 or 8 weeks) after the onset of each intervention of tooth brushing or professional cleaning.

Previous work has suggested that changes in levels of microbial and chemical biomarkers are indicative of future clinical patterns in health and disease (Teles et al., 2006; Offenbacher et al., 2010). A longer study beyond 8 weeks post intervention could provide further evidence to support these observed correlations between the clinical and subclinical metrics but would bring the associated risks of non-compliance with study protocols. The study subjects had mild-moderate gingivitis (overall mean GI at baseline = 1.0) and it must be recognised that they may not necessarily exhibit the most severe and profound inflammation at the test sites for chemical biomarker analysis.

2.8 Conclusion

The provision of sequential interventions, oral hygiene instruction with Sonicare *Flex*Care+ HX6942/20 (Philips Oral Healthcare Inc. Bothell, WA, USA) and subsequent professional prophylaxis brought about significant improvements in the clinical signs of mild to moderate gingivitis. A shift in the microbiological flora towards those species associated with health was observed following the interventions however there were no significant difference in local levels of biological markers of inflammation (cytokines, MMPs). The greatest change in clinical signs resulted from the instruction in self-performed plaque control with a powered toothbrush. This intervention was effective in plaque removal and therefore can be used to evaluate the effect that plaque control may have in a patient group with symptomatic gingival disease.

Chapter 3

A personalised plaque control programme for managing the gingival manifestations of oral lichen planus (Clinical Trial 2)

3.1 Introduction

Gingival manifestations are most commonly seen in the erosive, ulcerative and atrophic forms of oral lichen planus (Jadinski and Shklar, 1976, Scully and Porter, 1997, Stoopler et al., 2003, Leao et al., 2008, Lo Russo et al., 2009). The lesions are often symptomatic with the extent of gingival involvement varying from chronic epithelial desquamation, erythema, and erosion to blistering of the attached and marginal gingiva (Prinz, 1932, Scully and Porter, 1997, Guiglia et al., 2007).

Symptomatic ulcerative or erosive gingival lesions have the potential to compromise effective plaque control (Lo Russo *et al.*, 2009). It is recognised that whilst good plaque control does not bring about complete resolution, it may reduce the frequency of the symptoms of oral lichen planus (Erpenstein, 1985; Holmstrup *et al.*, 1990; Guiglia *et al.*, 2007; Lopez-Jornet and Camacho-Alonso, 2010a). Further, most guidelines and reviews have recommended that as part of the initial treatment, the optimisation of plaque control may also prevent periodontal damage (Lo Russo *et al.*, 2008).

Current evidence suggests that topical corticosteroids should be the first line treatment, but there is no universally agreed second line treatment such as short courses of systemic corticosteroids (Cribier *et al.*, 1998; Carrozzo and Gandolfo, 1999; Eisen, 2002; Eisen *et al.*, 2005; Lodi *et al.*, 2005b; Al-Hashimi *et al.*, 2007; Scully and Carrozzo, 2008; Carrozzo and Thorpe, 2009; BSOM, 2010; Cheng *et al.*, 2012). Factors that have been found to expedite improvement of symptomatic lesions include reassurance, avoidance of exacerbating factors such as certain foods, avoidance of smoking, alcohol and improving plaque control (Ramon-Fluixa *et al.*, 1999; Thongprasom *et al.*, 2003; Thongprasom *et al.*, 2011). The outcomes of the two most recent systematic reviews of interventions for treating oral lichen planus have recommended an evaluation of cost-effectiveness of treatments should also take place (Zakrzewska *et al.*, 2005; Lodi *et al.*, 2012).

3.2 Primary aim

The primary aim of this study was to evaluate the impact of a tailored oral hygiene programme for patients with gingival manifestations of oral lichen planus.

3.3 Secondary objectives

- To investigate the association in clinical outcome and oral health related quality of life following the intervention of a tailored hygiene programme.
- To investigate the change in the visual extent of the lesions (i.e. site and severity) following the intervention.
- To undertake a cost-benefit analysis of providing a personalised plaque control programme to patients with gingival manifestations of oral lichen planus.
- To compare the change in levels of biomarkers from baseline between the intervention and the control groups in a subset of the sampled cohort.

3.4 Materials and methods

This was a parallel group, longitudinal, randomised controlled trial (RCT). The intervention comprised an oral hygiene programme consisting of personalised advice using a powered toothbrush (Sonicare *Flex*Care HX6942/20, Philips Oral Healthcare, Bothell, WA, USA) and interdental cleaning aids. Study participants were evaluated at 4-weeks and 20-weeks following baseline and randomisation (Figure 25).

The study was conducted in accordance with ICH Good Clinical Practice (GCP) and a favourable ethical opinion was provided for the study by Sunderland Research Ethics Committee (REC), UK on September 27th 2010 (Ref. 10/H0904/48). The study was sponsored by the Joint Research and Development office, Newcastle upon Tyne NHS Foundation Trust. The study design was insured and risk assessed through Newcastle University with Zurich Municipal. In addition, Caldicott guardian approval was obtained from Newcastle upon Tyne NHS Foundation Trust.

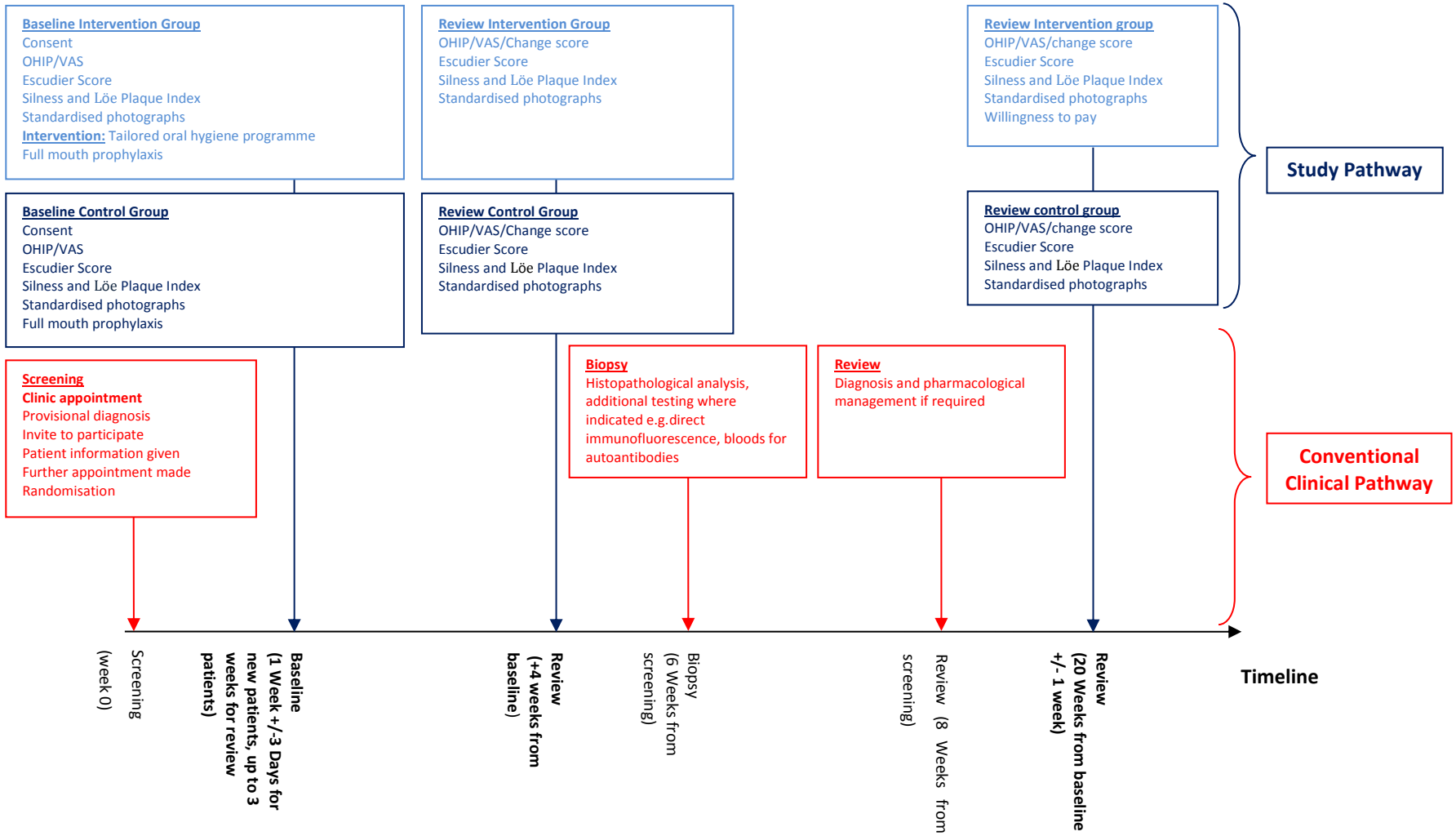


Figure 25. Clinical Trial 2. Study overview showing study and conventional treatment pathways and the timeline for appointments.

3.4.1 Subjects

Patients were recruited from consultant led oral medicine and periodontal clinics at Newcastle Dental Hospital between February 2011 and June 2012. Patients were provided with a REC approved information sheet (Appendix 4), and a further appointment was made or further time given to consider their involvement. All subjects whose diagnosis of oral lichen planus was not previously confirmed by biopsy or histopathological analysis had this performed, along with direct immunofluorescence and blood tests where appropriate (BSOM, 2010). Participation in the study was designed to fit within the patients' standard clinical care pathway (Figure 25).

The following inclusion and exclusion criteria were applied:

Inclusion criteria

- Adult patients aged 18 years and above;
- Willing and able to complete questionnaires;
- Able to provide informed consent to participate in the study;
- Patients newly referred to, or who are under review at Newcastle Dental Hospital with a desquamative gingivitis and a provisional diagnosis of oral lichen planus;

Exclusion criteria

- Unable to attend for the additional appointments prior to their biopsy;
- Unable to complete questionnaires; large print questionnaires were made available for those with visual impairment. Alternatively the questionnaire was read to the subject;
- Potential subjects that have been a participant in a research study within the previous 28 days;
- Potential subjects who were involved in other research studies at the time of recruitment.

3.4.2. Power and Sample Size

Sample size was determined using the OHIP as the primary outcome measure, with pain, clinical indices and cost-effectiveness being secondary outcomes. The minimally important difference, the smallest difference between groups of an outcome that patients perceive as having a beneficial effect, was used to calculate the standard deviation and subsequently the number of subjects required in the study (Allen *et al.*, 2009; John *et al.*, 2009). Powering the study at 80% using a standard deviation of 10.49, 49 subjects in each group were required to detect a

difference with confidence at the 95% level (Allen *et al.*, 2009; John *et al.*, 2009). The attrition rate was expected to be high (Hewitt *et al.*, 2010). To allow for 20% dropout rate (non-compliance with the protocol and loss to follow up) the *a priori* estimate of subjects to be recruited was 118. This calculation was independently verified.

This estimate uses figures from other oral conditions; the minimally important (clinical) difference for OHIP in patients with oral lichen planus is likely to be different. Therefore a post hoc calculation was planned to attain whether the study was appropriately powered.

3.4.3 Randomisation

Randomisation using sealed opaque envelopes was carried out in blocks of 10 to ensure roughly similar numbers of subjects in each group (Dallal, 2008; Suresh, 2011). These envelopes were opened in front of the subject by the researcher following consent and enrolment into the study and after the baseline records had been recorded.

3.4.4 Calibration of examiners

Calibration of the clinical examiner was undertaken using repeated measurements on subjects not involved with the study. A weighted Cohen's Kappa statistic was used to assess the agreement between two ratings after adjusting for chance (Cohen, 1968). The weighted Cohen's Kappa attained were: Plaque Index = 0.80 [95% CI 0.75, 0.84]; Escudier Index site score 0.96 [95%CI 0.83, 1.00] and activity score 0.78 [95%CI 0.63, 0.91].

3.4.5 Study visits

Visit 1

Written informed consent was obtained from each participant (Appendix 5). Subjects were asked to self-complete the OHIP questionnaire and 100mm VAS scale for pain. Clinical examination took place and Plaque Index (Silness and Löe, 1964) and Escudier oro-mucosal disease scores (Escudier *et al.*, 2007) were recorded along with clinical photographs.

At baseline, the intervention group received an oral hygiene programme comprising personalised oral hygiene advice using a powered toothbrush, Sonicare

FlexCare+ HX6942/20 (Philips Oral Healthcare Inc. Bothell, WA, USA) with instructions to brush for 2 minutes. They were also provided with interdental cleaning aids, either appropriately sized TePe® extra soft interdental brushes (TePe Munhygienprodukter, Sweden) ranging from ISO size 1-6 or Oral-B Dental Floss (Procter & Gamble, UK). Intervention group subjects were provided with all products required for the full duration of the study.

All subjects (control and intervention) received a prophylaxis at baseline (which did not include scaling or root planing) and were provided with standardised toothpaste (Pronamel®, GlaxoSmithKline, UK). Subjects were provided with sufficient products to last for the duration of the study.

For a small subgroup (n=12), GCF and unstimulated saliva samples were obtained to assess local levels of inflammatory biomarkers.

Saliva was collected by asking subjects to sit upright with their head inclined forwards. They were asked not to swallow any saliva or cough up any mucus during this collection period. Saliva was allowed to pool in a sterile polypropylene graduated collection tube until at least 3 ml was collected. The unstimulated saliva samples were centrifuged for 10 minutes at 3000g. After centrifugation, the resulting saliva supernatant was separated into 4 aliquot tubes with roughly equal volumes (approximately 0.5-0.9ml each).

Four gingival crevicular fluid samples were collected per participant at each visit (171/163/471/463) (Figure 8) using the same protocols and methods described in Chapter 2. Volumes were determined using a Periotron 8000 (Oraflow, Planview, New York, USA). All samples were snap-frozen and stored at -80°C before shipment by international courier in dry ice to the University of North Carolina (UNC) cytokine analysis facility.

Visit 2

Visit 2 took place 4 weeks +/- 1 week after baseline. Subjects were asked to self-complete the OHIP questionnaire, VAS scale for pain and a global change score (improved a lot, improved slightly, stayed the same, become slightly worse, become a lot worse). Clinical assessment using the Plaque Index (Silness and Löe, 1964), Escudier oro-mucosal disease score (Escudier *et al.*, 2007) and clinical

photographs were recorded. For the subgroup (n=12) GCF and saliva samples were also taken.

Visit 3

Visit 3 took place 20 weeks +/- 1 week after baseline. Subjects were asked to self-complete the OHIP questionnaire, VAS scale for pain and a global change score. Clinical assessment using the Plaque Index (Silness and Löe, 1964), Escudier oro-mucosal disease score (Escudier *et al.*, 2007) and clinical photographs were recorded.

At this visit recruits to the intervention group were asked to complete a short questionnaire, which recorded out of pocket payments and lost work time relating to the care provided during the study. They were also asked to state the maximum amount they would be willing to pay to purchase the powered toothbrush and advice in an open-ended valuation exercise. The valuation was preceded by the patients being given cards representing a range of prices [£1 to £2000] and asked to consider whether they would pay the amount listed on each card. This exercise is frequently undertaken as an aid to the valuation of health services in contingent valuation studies.

3.4.6 Biomarker analysis

Testing of the saliva and GCF samples was performed using multiplex (BioPlex 200) analysis using kits from R&D Systems (Minneapolis, MN), according to the manufacturer's instructions, previously detailed in Chapter 2. On completion of all study sample collections and shipping to the UNC facility, the samples were readied for analysis by thawing and again centrifuging the thawed materials, as recommended by Salimetrics, Inc. for optimal preparation of samples for analysis of salivary analytes. Tests to be performed included subsets of the Cytokine Panel A by R&D systems (Minneapolis, MN): IL-1β, IL-2, MIP-1α MIP-1β and RANTES and the MMP panel (MMP-1, MMP-3, MMP-8, MMP-9, MMP-13).

3.5 Data management and confidentiality

The study records, including each participant's signed informed consent and other documents pertaining to the conduct of the study were retained and kept in a secure area. Participant information was kept confidential and individual subject records contained sufficient data only to allow identification of the participants throughout the study. Clinical record forms were coded and anonymised, and each participant was assigned a study number. The master list with subject identifiers was retained.

3.6 Reimbursement

The oral hygiene aids including the powered toothbrush were provided to the intervention group to keep; control patients received the same powered toothbrush at the end of the study. Reasonable travel expenses were reimbursed to participants attending visits above their routine care pathway upon production of receipts.

3.7 Substantial amendments

Two substantial amendments were applied for to Sunderland REC. These were given favourable opinions by a sub-committee of the Sunderland REC on 4th February 2011 and 30th September 2011. The first amendment was to increase the time from recruitment to Visit 1 for review patients (not new patients) from 1 week to 3 weeks in order to maximise recruitment. The second amendment was to collect GCF and saliva samples from a subgroup of intervention and control subjects.

3.8 Statistical and analytic plan

Unless otherwise noted, continuous variables were summarized using the number of observations, mean, standard deviation, median, minimum, and maximum. Categorical variables were summarized using the frequency count and the percentage of subjects in each category. Subjects were grouped according to the treatment received.

3.8.1 Analysis Populations

The population to be analysed was the modified-intention-to-treat (MITT) population. This comprised all randomized subjects with a baseline and at least one post-baseline OHIP evaluation. Subject 80 in the intervention group, Subjects 7 and 56 in control group only had baseline OHIP, so they were not included in the MITT population. 79 out of the 82 participants recruited were analysed.

	Intervention (N=39)	Control (N=43)	Cohort (N=82)
Randomized	39 (100%)	43 (100%)	82 (100%)
Modified-Intent-to-Treat (MITT)	38 (97%)	41 (95%)	79 (96%)

Table 29. Clinical Trial 2. Populations included in analysis.

3.8.2 Analysis of demographic and baseline characteristics

Standard subject demographics (e.g., age and gender) and baseline characteristics were summarized for all subjects randomized and for MITT subjects. For continuous subject characteristics, means were compared using one-way analysis of variance (ANOVA). The incidence of the categorical variables was compared using the Chi-square test or Fisher's exact test as appropriate. All summaries were presented by treatment group and overall.

3.8.3 Primary outcome analysis

The primary outcome measure for this study was overall sum OHIP score and by domain. The primary outcome measure was treated as a continuous variable. Analysis of OHIP score was performed on increase, decrease and net change from baseline at week 4 and week 20.

Increase was defined as the number of impacts reported at follow-up that were not reported at baseline. It was calculated as the sum of change from baseline for the questions with increased score. Increase quantifies only deterioration in quality of life, ignoring any decreases (Slade, 1998).

Decrease was defined as the number of impacts reported at baseline that were not reported at follow-up. It was calculated as the sum of reduction from baseline for the questions with decreased score. Decrease quantifies only improvements in quality of life, ignoring any increases (Slade, 1998).

Net change was defined as the number of impacts reported at follow-up minus the number of impacts at baseline. It was calculated as the sum of change from baseline for all questions. Arithmetically, this was equivalent to the increase minus decrease (Slade, 1998).

Statistical analysis of the data including parameter and confidence interval (CI) estimation was accomplished using SAS and STATA software. Comparisons between the treatment groups were performed using the F-Test. The following model was used:

$$y_{ij} = \mu + \alpha_i + W_j + \varepsilon_{ij}$$

Where:

y_{ij} Post baseline measurement for subject ($_i$) and treatment ($_j$);

μ Overall mean;

α_i Random subject effect;

W_j Extent to which change from baseline is attributable to treatment;

ε_i Unexplained/residual variation for each subject ($_i$) and treatment ($_j$).

Analysis was performed for increase, decrease, and net change of OHIP scores (ordinal and dichotomous) by domain and visit.

The biomarker data were summarised using descriptive statistics for the observed values and the changes from baseline. Two sided t-tests, without adjustment for multiple comparisons were used to assess the statistical significance of changes from baseline.

Finally, effect size (Cohen's *d*) was calculated from the mean pre and post-treatment scores for each group for each outcome measure. The purpose of this descriptive statistic is to measure the strength of a treatment effect and to

complement the comparisons made using the ANOVA statistics. It also allows comparisons between studies and meta-analyses to be more easily conducted.

Cohen's $d = \frac{x_i - x_{ii}}{s}$

x_i = mean pre-treatment.

x_{ii} = mean post-treatment.

S = standard deviation of pre-treatment.

3.8.4 Secondary efficacy analysis

Analyses similar to the primary analysis were performed for change from baseline in VAS (pain), Plaque Index, Mucosal Disease Score (i.e. total site score, severity score and activity score), GCF and saliva sample biomarker concentration at Week 4 and Week 20.

For biomarkers, the concentration was measured multiple times from subsamples. The mean concentration among subsamples was used for analysis. Log transformation was applied to biomarkers due to deviation from normality.

Spearman correlation coefficient was calculated between the OHIP and VAS, Plaque Index, Mucosal Disease Score and biomarkers. The analysis was applied to the results and change from baseline (net change for OHIP).

3.9 Results

Recruitment for the study ran from February 2011 to June 2012. 120 patients who attended oral medicine consultant diagnostic clinics were invited to participate. 82 accepted and were enrolled (39 intervention and 43 control subjects). It was not possible to recruit 98 subjects without extending the recruitment phase to beyond 17 months and allowing for the 20-week follow-up period.

3 intervention subjects and 2 control subjects were lost to follow-up. One subject failed to attend Visit 2 but was retained through to Visit 3.

The age of the participants ranged from 31 to 83 years at the time of enrolment, descriptive statistics are provided in Table 30. There were no significant differences in the treatment groups for demographic characteristics and baseline characteristics. The mean age for all randomized subjects was 61.4 years. The study included 15 (18.3%) males and 67 (81.7%) females reflecting the greater number of females with oral lichen planus in the wider population. Recruitment selection bias was not considered to be significant: the gender demographics of subjects that declined participation or were unable to fulfil the inclusion and exclusion criteria were similar to the study participants with 5 (15.6%) males and 27 (84.3%) females.

A summary of the baseline characteristics for the clinical and self-reported outcome measures are detailed in Table 31. There were no significant differences between intervention and control groups at baseline ($p>0.05$).

Outcome	Category	Group			P-value[a]
		Intervention (N=39)	Control (N=43)	Cohort (N=82)	
Age (years)	Mean (SD)	61.2 (9.90)	61.6 (11.80)	61.4 (10.90)	0.986
	95% CI	(58, 64.4)	(58, 65.2)	(59, 63.8)	
	Median	63	63	63	
	Min, Max	(39, 83)	(31, 83)	(31, 83)	
Gender	Gender 1	6 (15.4%)	9 (20.9%)	15 (18.3%)	0.810
	Gender 2	33 (84.6%)	34 (79.1%)	67 (81.7%)	

Table 30. Clinical Trial 2. Descriptive statistics showing the age demographics for the Intervention and Control groups.
[a]P-value is calculated by T-test for continuous variables, and Chi-squared test for categorical variables.

Outcome	Category	Group Intervention (N=39)	Control (N=43)	Cohort (N=82)	P-value[a]
OHIP Sum, Ordinal	Mean (SD) 95% CI Median Min, Max	49.5 (24.60) 41.5, 57.4 44 3, 92	48.7 (29.30) 39.6, 57.7 45 8, 158	49.1 (27.00) 43.1, 55.0 44.5 3, 158	0.991
OHIP Sum, Dichotomous	Mean (SD) 95% CI Median Min, Max	6.6 (4.90) 5.1, 8.2 6 0, 20	6.5 (6.90) 4.4, 8.6 4 0, 37	6.6 (6.00) 5.3, 7.9 5.5 0, 37	0.995
VAS	Mean (SD) 95% CI Median Min, Max	3.3 (2.10) 2.7,4.0 3.0 0, 7.2	3.4 (2.20) 2.7, 4.0 2.9 0, 8.1	3.4 (2.10) 2.9, 3.8 2.9 0, 8.1	0.999
Plaque Index	Mean (SD) 95% CI Median Min, Max	1.4 (0.40) 1.3, 1.5 1.4 0.7, 2.4	1.4 (0.30) 1.3, 1.6 1.4 0.9, 2.2	1.4 (0.30) 1.4, 1.5 1.4 0.7, 2.4	0.938
Mucosal Disease Score: **Site Score**	Mean (SD) 95% CI Median Min, Max	10.9 (2.50) 10.1, 11.7 11 7, 17	10.3 (2.20) 9.7, 11.0 10 7, 15	10.6 (2.40) 10.1, 11.1 10 7, 17	0.611
Mucosal Disease Score: **Severity Score**	Mean (SD) 95% CI Median Min, Max	14.9 (5.60) 13.1, 16.7 13 6, 29	12.9 (4.30) 11.5, 14.2 13 3, 21	13.8 (5.10) 12.7, 15.0 13 3, 29	0.181
Mucosal Disease Score: **Activity Score**	Mean (SD) 95% CI Median Min, Max	16.8 (7.10) 14.5, 19.1 15 6, 35	14 (5.30) 12.4, 15.6 14 3, 27	15.3 (6.30) 13.9, 16.7 14 3, 35	0.123

Table 31. Clinical Trial 2. Summary of baseline characteristics for all randomised subjects.
[a]P-value is calculated by T-test for continuous variables, and Chi-squared test for categorical variables.

3.9.1 Oral health impact profile

The two groups, intervention and control had a similar distribution of scores at baseline. Descriptive statistics for the sum ordinal scores are presented in Table 32 and dichotomous sum scores are presented in Table 33.

The groups had similar baseline mean OHIP ordinal sum scores 49.66 (ordinal) and 6.55 (dichotomous) for the intervention group and 49.39 (ordinal) and 6.71 (dichotomous) for the control group. The relationship between baseline and post-baseline for overall sum OHIP ordinal scores is presented in Figure 26 and overall sum dichotomous scores in Figure 27. In these reference plots, scores plotted below the reference line indicate an increase post-baseline compared to baseline. Values above the reference line indicate a decrease compared to baseline. Box plots were also produced for the ordinal sum scores (Figure 28) and for the dichotomous sum score (Figure 29). At week 4 and week 20 the distributions shift with both groups showing a reduction in OHIP ordinal scores overall. The shift for the intervention group was more than the control group.

Least square (LS) mean and statistical analysis are presented in Table 34 for the sum ordinal scores and Table 35 for the sum dichotomous scores.

Both treatment groups contained subjects who experienced an increase in OHIP ordinal and dichotomous scores. The increase in ordinal scores indicates subjects experiencing deterioration to some questions (impact started or happened more frequently from baseline ignoring the questions with improvement). The increase in dichotomous scores indicates some impacts happened “fairly often” or “very often” at post-baseline, which did not happen at such frequency at baseline (ignoring any improvement). The intervention group experienced less increase in both ordinal and dichotomous scores in overall domain. In each domain, the increase for the intervention group is similar or less than the control group.

Both treatment groups contained subjects who experienced a decrease in OHIP ordinal and dichotomous scores. The decrease in ordinal scores indicates subjects experiencing improvement to some questions (impact stopped or happened less frequently from baseline ignoring the questions with deterioration). The decrease in dichotomous scores indicates some impacts happened “fairly often” or “very often” at baseline did not happen at such frequency post-baseline. The intervention

group experienced greater decrease in both ordinal and dichotomous scores overall. In each domain, the decrease for intervention group is similar or more than the control group.

Both treatment groups experienced negative net change (post-baseline minus baseline) in OHIP ordinal and dichotomous scores. The negative net change in ordinal scores indicates subjects experiencing an overall improvement in given domain, taking into account both the improvement and deterioration to individual questions. The negative net change in dichotomous scores indicates an overall decrease in the impacts that happened "fairly often" or "very often" from baseline to post-baseline, taking into account some impacts happened more frequently, and some happened less frequently. The intervention group experienced greater negative net change in both ordinal and dichotomous scores in overall domain, indicating improvements in oral health related quality of life.

Examination of the individual domain scores provides further insight into the range of impacts that oral lichen planus has on a subject's quality of life. Statistical output for each domain was undertaken using ordinal coding and presented as follows:

- Functional limitation Table 36
- Physical pain Table 37
- Psychological discomfort Table 38
- Physical disability Table 39
- Psychological disability Table 40
- Social disability Table 41
- Handicap Table 42

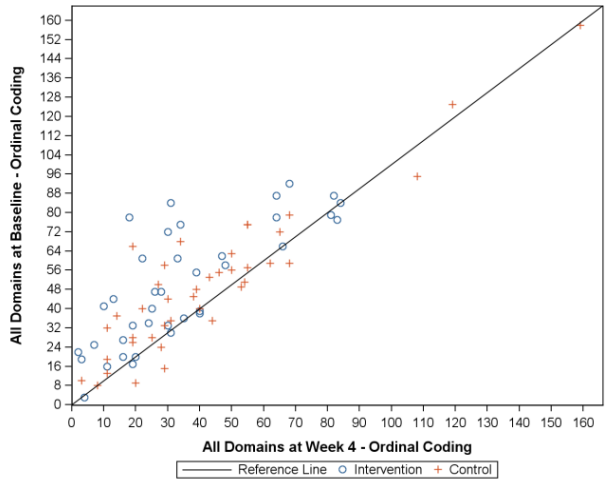
Graphical representation using bar charts of the domain scores are presented in Figure 30 and for net change from baseline at 4 and 20 weeks in Figure 31. Those domains with significant differences between groups at 4 and 20 weeks were functional limitation (p=0.0216, p=0.0137), psychological discomfort (p=0.007, p=0.0016) and physical disability (p=0.0137, p=0.0035). The psychological disability domain showed significant differences at the 20-week (p=0.003) but not the 4-week follow up (p=0.435). There were no significant correlations in the social disability (p=0.7628, 0.8106) and handicap domains (p=0.8575, p=0.2239) and a constant although not statistically significant association (p=0.0594, p=0.0522) in the physical pain domain.

Visit	Outcome	Treatment	n	Mean (SD)	Median	Min, Max
Baseline	Result	Intervention	38	49.66 (24.86)	45.5	3, 92
		Control	41	49.39 (29.82)	48	8, 158
Week 4	Result	Intervention	38	34.55 (23.84)	30	2, 84
		Control	40	42.25 (31.03)	36	3, 159
	Increase	Intervention	32	6.34 (5.28)	5	1, 21
		Control	37	7.54 (5.32)	7	1, 19
	Decrease	Intervention	38	20.45 (13.90)	17.5	1, 62
		Control	38	15.29 (9.61)	13	2, 49
	Net Change	Intervention	38	-15.11 (16.31)	-14	-60, 6
		Control	40	-7.55 (12.87)	-7	-47,14
Week 20	Result	Intervention	36	31.64 (23.86)	27.5	1, 88
		Control	41	41.66 (28.87)	40	4, 162
	Increase	Intervention	28	6.54 (5.36)	5.5	1, 24
		Control	37	10.30 (7.75)	9	1, 34
	Decrease	Intervention	36	24.56 (15.90)	23	1, 71
		Control	40	17.45 (12.24)	16	1, 57
	Net Change	Intervention	36	-19.47 (18.84)	-15.5	-70, 11
		Control	41	-7.73 (17.39)	-7	-47, 32

Table 32. Clinical Trial 2. Oral Health Impact Profile (OHIP) scores. Descriptive summary all domains, **ordinal coding**, MITT Subjects.
The intervention and control groups were comparable at baseline exhibiting similar impacts recorded by OHIP. Both groups showed both increases and decreases in OHIP ordinal sum score at follow-up visits with the net result being a decrease in OHIP ordinal sum score overall for both groups.

Visit	Outcome	Treatment	n	Mean (SD)	Median	Min, Max
Baseline	Result	Intervention	38	6.55 (4.91)	6	0, 20
		Control	41	6.71 (6.97)	5	0, 37
Week 4	Result	Intervention	38	3.03 (3.28)	2	0, 11
		Control	40	5.30 (7.53)	3	0, 41
	Increase	Intervention	11	1.73 (1.56)	1	1, 6
		Control	23	1.96 (1.52)	1	1, 6
	Decrease	Intervention	35	4.37 (3.28)	4	1, 17
		Control	31	3.39 (2.11)	3	1, 9
	Net Change	Intervention	38	-3.53 (3.56)	-3	-17, 3
		Control	40	-1.50 (3.02)	-1.5	-9, 6
Week 20	Result	Intervention	36	2.56 (3.32)	1	0, 12
		Control	41	4.90 (7.17)	3	0, 41
	Increase	Intervention	11	2.09 (1.38)	2	1, 5
		Control	22	2.45 (1.63)	2	1, 6
	Decrease	Intervention	33	5.36 (3.95)	5	1, 20
		Control	32	4.00 (3.45)	3	1, 16
	Net Change	Intervention	36	-4.28 (4.28)	-3.5	-19, 2
		Control	41	-1.80 (4.24)	-1	-14, 6

Table 33. Clinical Trial 2. Oral Health Impact Profile (OHIP) scores. Descriptive summary all domains, **dichotomous coding**, MITT Subjects.
The intervention and control groups were comparable at baseline exhibiting similar impacts recorded by OHIP. Both groups showed both increases and decreases in OHIP dichotomous sum score at follow-up visits with the net result being a decrease in OHIP dichotomous sum score overall for both groups.



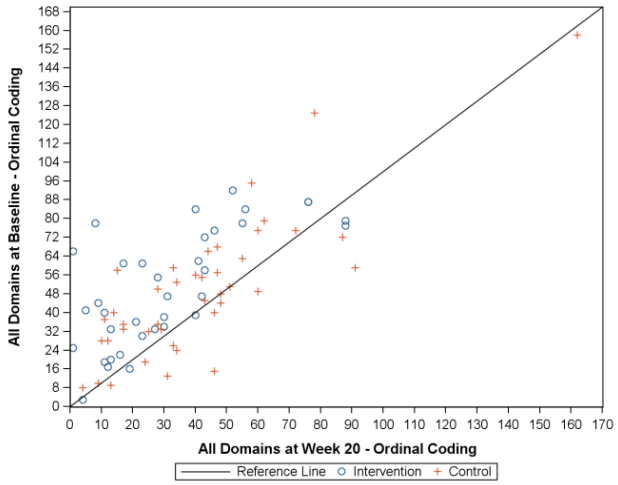
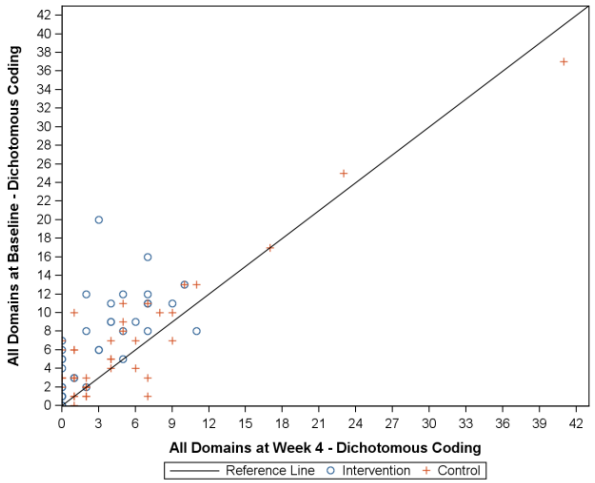


Figure 26. Clinical Trial 2. Reference Plot: Oral Health Impact Profile (OHIP) **ordinal coding**, all domains. MITT Subjects.



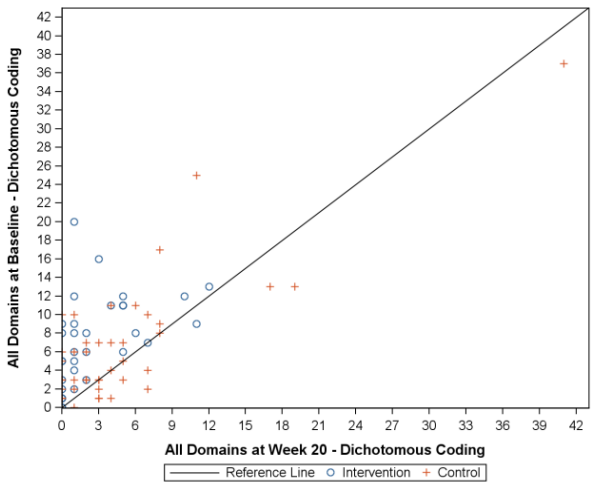


Figure 27. Clinical Trial 2. Reference plot: Oral Health Impact Profile (OHIP) **dichotomous coding**, all domains. MITT subjects.

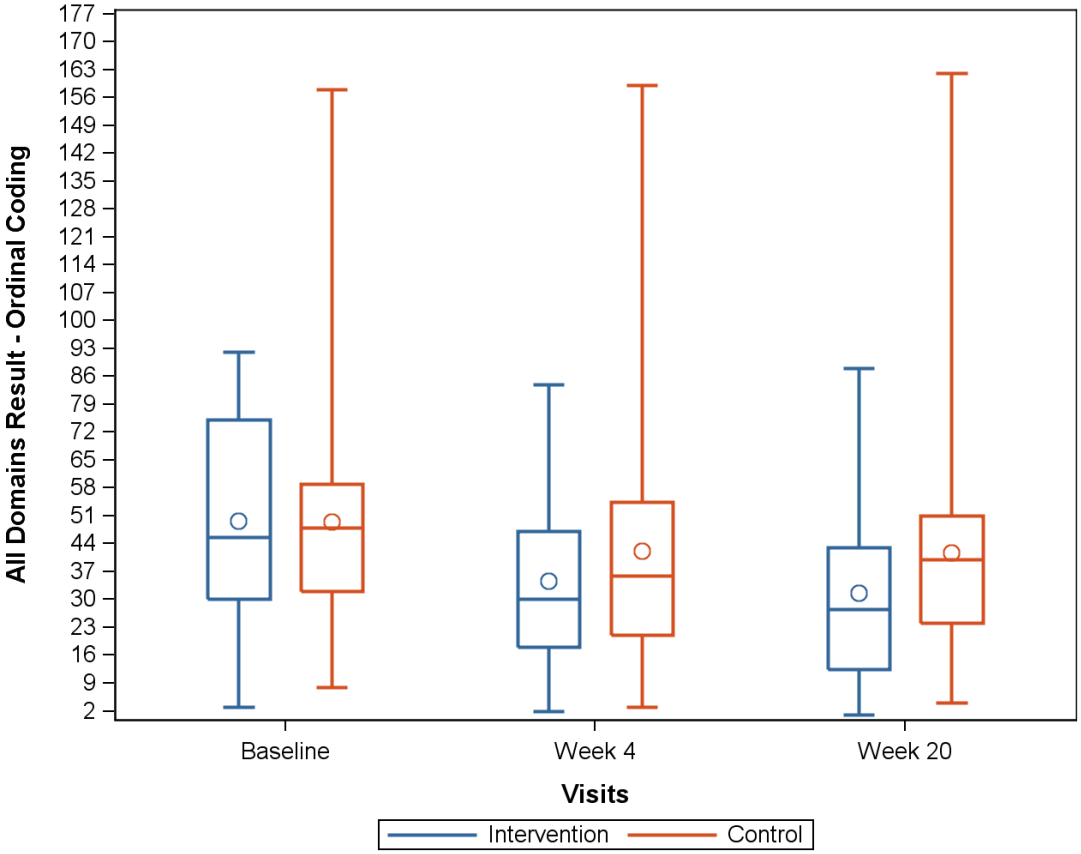


Figure 28. Clinical Trial 2. Box Plot: Oral Health Impact Profile Questionnaire (OHIP) scores. All domains, **ordinal coding**, MITT Subjects.
Ordinal is based on the sum of scores of each question original category (never =0, hardly ever=1, occasionally=2, fairly often=3, very often=4). At the 4- and20-week follow-up, both groups show improvement in OHIP ordinal scores. The differences between the group means were statistically significant at follow-up (ANOVA p=0.9657 at baseline; p=0.0215 at week 4; p=0.0044 at week 20).

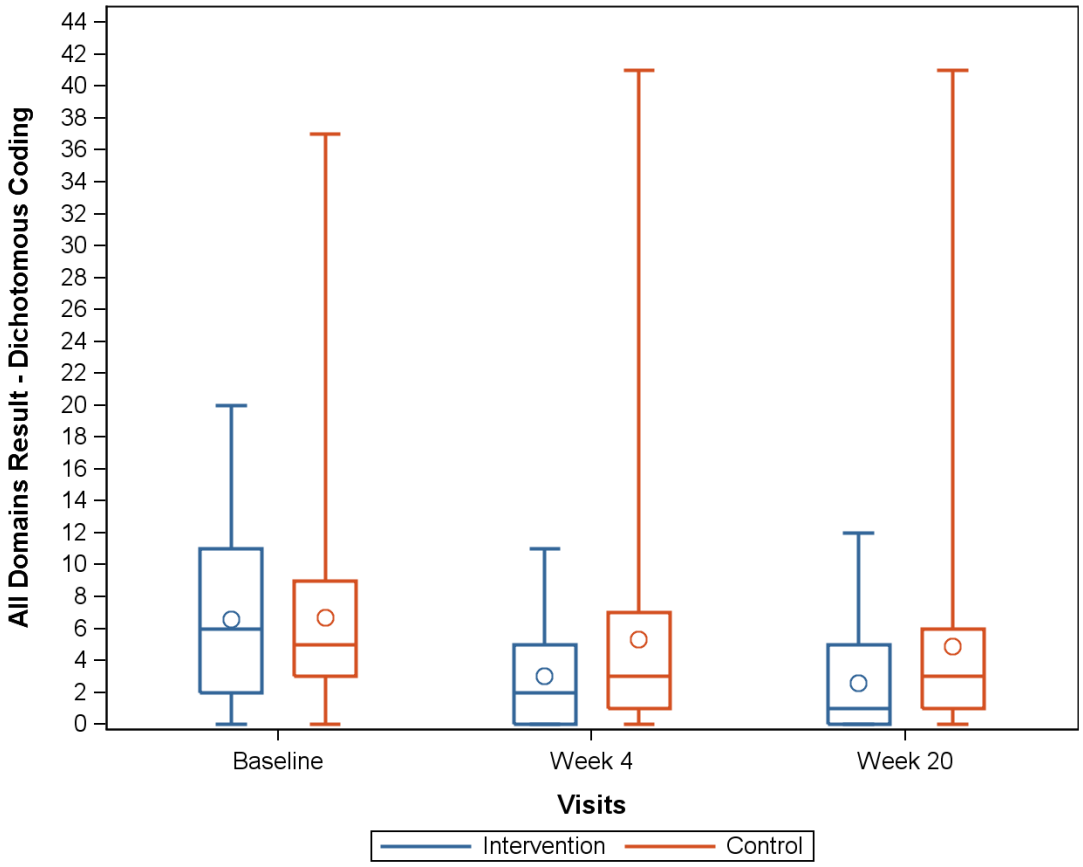


Figure 29. Clinical Trial 2. Box Plot: Oral Health Impact Profile (OHIP) scores. All domains, **dichotomous coding**, MITT Subjects.
Dichotomous is based on the sum of score where each question is rated as 0 or 1 (occasionally, hardly ever, or never=0; fairly often or very often=1). At the 4- and20-week follow-up, both groups show improvement in OHIP dichotomous scores. The differences between the group means were statistically significant at follow-up (ANOVA p=0.9101 at baseline; p=0.0045 at week 4; p=0.0072 at week 20).

Visit	Outcome	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	49.66 (4.47)	49.39 (4.30)	0.27 (6.20)	0.9657
		95% CI	40.76, 58.56	40.82, 57.96	-12.09, 12.62	
Week 4	Result	n	38	40		
		LS Mean (SE)	34.62 (2.31)	42.19 (2.25)	-7.57 (3.23)	0.0215
		95% CI	30.01, 39.22	37.70, 46.68	-14.00, -1.15	
	Increase	n	38	40		
		LS Mean (SE)	6.35 (0.92)	7.53 (0.86)	-1.18 (1.26)	0.3517
		95% CI	4.50, 8.20	5.82, 9.25	-3.71, 1.34	
	Decrease	n	38	40		
		LS Mean (SE)	20.65 (1.79)	15.09 (1.79)	5.56 (2.54)	0.0315
		95% CI	17.08, 24.22	11.51, 18.66	0.51, 10.62	
	Net Change	n	38	40		
		LS Mean (SE)	-15.12 (2.31)	-7.54 (2.25)	-7.57 (3.23)	0.0215
		95% CI	-19.72, -10.51	-12.03, -3.05	-14.00, -1.15	
Week 20	Result	n	36	41		
		LS Mean (SE)	30.95 (2.81)	42.26 (2.63)	-11.31 (3.85)	0.0044
		95% CI	25.35, 36.55	37.02, 47.51	-18.99, -3.64	
	Increase	n	36	41		
		LS Mean (SE)	6.53 (1.30)	10.30 (1.13)	-3.77 (1.72)	0.0319
		95% CI	3.94, 9.12	8.05, 12.56	-7.21, -0.34	
	Decrease	n	36	41		
		LS Mean (SE)	24.46 (2.05)	17.54 (1.94)	6.92 (2.82)	0.0165
		95% CI	20.38, 28.54	13.67, 21.40	1.30, 12.54	
	Net Change	n	36	41		
		LS Mean (SE)	-19.25 (2.81)	-7.93 (2.63)	-11.31 (3.85)	0.0044
		95% CI	-24.84, 13.65	-13.18, -2.69	-18.99, -3.64	

Table 34. Clinical Trial 2. Oral Health Impact Profile (OHIP) scores. Least squares means of all domains, **ordinal coding**, MITT subjects.
Ordinal is based on the sum of scores of each question (never =0, hardly ever=1, occasionally=2, fairly often=3, very often=4). ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error.
[a]P-value is based on a mixed model F-test (Ho: Both treatments equal). [b] Diff = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Outcome	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	6.55 (0.98)	6.71 (0.95)	-0.15 (1.37)	0.9101
		95% CI	4.59, 8.51	4.82, 8.59	-2.87, 2.57	
Week 4	Result	n	38	40		
		LS Mean (SE)	3.13 (0.51)	5.20 (0.49)	-2.07 (0.71)	0.0045
		95% CI	2.12, 4.14	4.22, 6.18	-3.48, -0.66	
	Increase	n	38	40		
		LS Mean (SE)	1.73 (0.45)	1.96 (0.31)	-0.23 (0.55)	0.6822
		95% CI	0.81, 2.65	1.32, 2.59	-1.35, 0.90	
	Decrease	n	38	40		
		LS Mean (SE)	4.47 (0.43)	3.28 (0.45)	1.18 (0.62)	0.0621
		95% CI	3.61, 5.32	2.38, 4.19	-0.06, 2.43	
	Net Change	n	38	40		
		LS Mean (SE)	-3.55 (0.51)	-1.48 (0.49)	-2.07 (0.71)	0.0045
		95% CI	-4.56, -2.54	-2.46, -0.49	-3.48, -0.66	
Week 20	Result	n	36	41		
		LS Mean (SE)	2.51 (0.64)	4.94 (0.60)	-2.43 (0.88)	0.0072
		95% CI	1.23, 3.79	3.74, 6.14	-4.19, -0.68	
	Increase	n	36	41		
		LS Mean (SE)	2.01 (0.45)	2.50 (0.32)	-0.49 (0.55)	0.3869
		95% CI	1.09, 2.93	1.85, 3.15	-1.62, 0.64	
	Decrease	n	36	41		
		LS Mean (SE)	5.40 (0.53)	3.96 (0.54)	1.45 (0.75)	0.0600
		95% CI	4.35, 6.46	2.88, 5.03	-0.06, 2.96	
	Net Change	n	36	41		
		LS Mean (SE)	-4.26 (0.64)	-1.82 (0.60)	-2.43 (0.88)	0.0072
		95% CI	-5.54, -2.98	-3.02, -0.62	-4.19, -0.68	

Table 35. Clinical Trial 2. Oral Health Impact Profile (OHIP) scores. Least squares means of all domains, **dichotomous coding**, MITT subjects.
Dichotomous is based on the sum of score where each question is rated as 0 or 1 (occasionally, hardly ever, or never=0; fairly often or very often=1). ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error. [a]P-value is based on a mixed model F-test (Ho: Both treatments equal). [b] Diff = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Outcome	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	10.53 (0.87)	9.80 (0.83)	0.72 (1.20)	0.5503
		95% CI	8.80, 12.25	8.14,11.47	-1.67, 3.12	
Week 4	Result	n	38	40		
		LS Mean (SE)	7.71 (0.52)	9.40 (0.50)	-1.69 (0.72)	0.0216
		95% CI	6.68. 8.74	8.40, 10.40	-3.12, -0.25	
Week 20	Result	n	36	41		
		LS Mean (SE)	6.95 (0.55)	8.85 (0.51)	-1.89 (0.75)	0.0137
		95% CI	5.87. 8.04	7.83, 9.87	-3.39, -0.40	

Table 36. Clinical Trial 2. **Functional limitation domain**. OHIP ordinal sum scores, MITT subjects.

This domain contains the OHIP questions relating to: difficulty chewing, trouble pronouncing words, teeth that don't look right, appearance affected, stale breath, taste, food catching, digestion, and dentures not fitting.
ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error. [a]P-value is based on a mixed model F-test (Ho: Both treatments equal). [b] Diff = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Outcome	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	13.68 (0.88)	14.34 (0.85)	-0.66 (1.23)	0.5934
		95% CI	11.93, 15.44	12.65, 16.03	-3.10, 1.78	
Week 4	Result	n	38	40		
		LS Mean (SE)	9.63 (0.65)	11.38 (0.64)	-1.75 (0.91)	0.0594
		95% CI	8.33, 10.93	10.11, 12.64	-3.56, 0.07	
Week 20	Result	n	36	41		
		LS Mean (SE)	9.68 (0.77)	11.77 (0.72)	-2.09 (1.06)	0.0522
		95% CI	8.14, 11.22	10.33, 13.21	-4.20, 0.02	

Table 37. Clinical Trial 2. **Physical pain domain**. OHIP ordinal sum scores, MITT subjects.

This contains the OHIP questions relating to: painful aching, sore jaw, headaches, sensitive teeth, toothache, painful gums, eating comfort, sore spots, discomfort (dentures).
ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error. [a]P-value is based on a mixed model F-test (Ho: Both treatments equal). [b] Difference = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Outcome	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	7.79 (0.83)	7.93 (0.80)	-0.14 (1.15)	0.9054
		95% CI	6.14, 9.44	6.34, 9.52	-2.43, 2.16	
Week 4	Result	n	38	40		
		LS Mean (SE)	4.84 (0.48)	6.68 (0.46)	-1.84 (0.66)	0.0070
		95% CI	3.89, 5.79	5.76, 7.60	-3.17, -0.52	
Week 20	Result	n	36	41		
		LS Mean (SE)	4.12 (0.56)	6.63 (0.52)	-2.51 (0.77)	0.0016
		95% CI	3.01, 5.23	5.58, 7.67	-4.04, -0.98	

Table 38. Clinical Trial 2. **Psychological discomfort domain**. OHIP ordinal sum scores, MITT subjects.

This contains the OHIP questions relating to: being worried, self-conscious, miserable, appearance and tension. ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error. [a]P-value is based on a mixed model F-test (Ho: Both treatments equal). [b] Difference = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Outcome	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	7.97 (0.91)	7.51 (0.88)	0.46 (1.27)	0.7163
		95% CI	6.16, 9.79	5.76, 9.26	-2.06, 2.98	
Week 4	Result	n	38	40		
		LS Mean (SE)	5.36 (0.57)	7.38 (0.56)	-2.03 (0.80)	0.0137
		95% CI	4.21, 6.50	6.27, 8.50	-3.63, -0.43	
Week 20	Result	n	36	41		
		LS Mean (SE)	4.65 (0.61)	7.18 (0.57)	-2.53 (0.84)	0.0035
		95% CI	3.44, 5.87	6.04, 8.32	-4.20, -0.86	

Table 39. Clinical Trial 2. **Physical disability domain**. OHIP ordinal sum, MITT subjects.

This contains the OHIP questions relating to: clarity of speech, being misunderstood, flavour in food, ability to brush teeth, avoidance of eating, diet, inability to eat (dentures), avoidance of smiling, interruption of meals.
ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error. [a]P-value is based on a mixed model F-test (Ho: Both treatments equal). [b] Difference = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Outcome	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	5.76 (0.79)	5.68 (0.76)	0.08 (1.10)	0.9418
		95% CI	4.19, 7.33	4.17, 7.20	-2.10, 2.26	
Week 4	Result	n	38	40		
		LS Mean (SE)	3.99 (0.44)	4.48 (0.43)	-0.49 (0.62)	0.4349
		95% CI	3.11, 4.88	3.62, 5.34	-1.71, 0.75	
Week 20	Result	n	36	41		
		LS Mean (SE)	3.21 (0.56)	4.89 (0.53)	-1.68 (0.77)	0.0324
		95% CI	2.08, 4.33	3.84, 5.94	-3.22, -0.15	

Table 40. Clinical Trial 2. **Psychological disability domain**. OHIP ordinal sum scores, MITT subjects.

This contains the OHIP questions relating to: interruption of sleep, being upset, difficulty in relaxing, depressed, concentration being affected and embarrasment.
ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error. [a]P-value is based on a mixed model F-test (Ho: Both treatments equal). [b] Difference = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Outcome	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	1.39 (0.49)	1.90 (0.47)	-0.51 (0.68)	0.4584
		95% CI	0.42, 2.37	0.96, 2.84	-1.86, 0.85	
Week 4	Result	n	38	40		
		LS Mean (SE)	1.41 (0.24)	1.31 (0.23)	0.10 (0.33)	0.7628
		95% CI	0.94, 1.89	0.85, 1.77	-0.56, 0.77	
Week 20	Result	n	36	41		
		LS Mean (SE)	1.26 (0.29)	1.16 (0.27)	0.10 (0.40)	0.8106
		95% CI	0.68, 1.83	0.62, 1.70	-0.69, 0.89	

Table 41. Clinical Trial 2. **Social disability domain**. OHIP ordinal sum scores, MITT subjects.

This contains the OHIP questions relating to: avoid going out, being less tolerant of others, trouble getting on with others, Irritability with others, difficulty doing jobs.
ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error. [a]P-value is based on a mixed model F-test (Ho: Both treatments equal). [b] Difference = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Outcome	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	2.53 (0.58)	2.22 (0.56)	0.31 (0.81)	0.7045
		95% CI	1.37, 3.68	1.11, 3.33	-1.30, 1.91	
Week 4	Result	n	38	40		
		LS Mean (SE)	1.65 (0.29)	1.58 (0.28)	0.07 (0.40)	0.8575
		95% CI	1.08, 2.22	1.02, 2.14	-0.72, 0.87	
Week 20	Result	N	36	41		
		LS Mean (SE)	1.17 (0.32)	1.70 (0.30)	-0.53 (0.43)	0.2239
		95% CI	0.54, 1.80	1.11, 2.29	-1.39, 0.33	

Table 42. Clinical Trial 2. **Handicap domain**. OHIP ordinal sum scores, MITT subjects.

This contains the OHIP questions relating to: health worsened, financial loss, inability to enjoy people's company, unsatisfying life, inability to function, inability to work.
ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error. [a]P-value is based on a mixed model F-test (Ho: Both treatments equal). [b] Difference = Mean (SD) of the treatment difference (Intervention - Control).

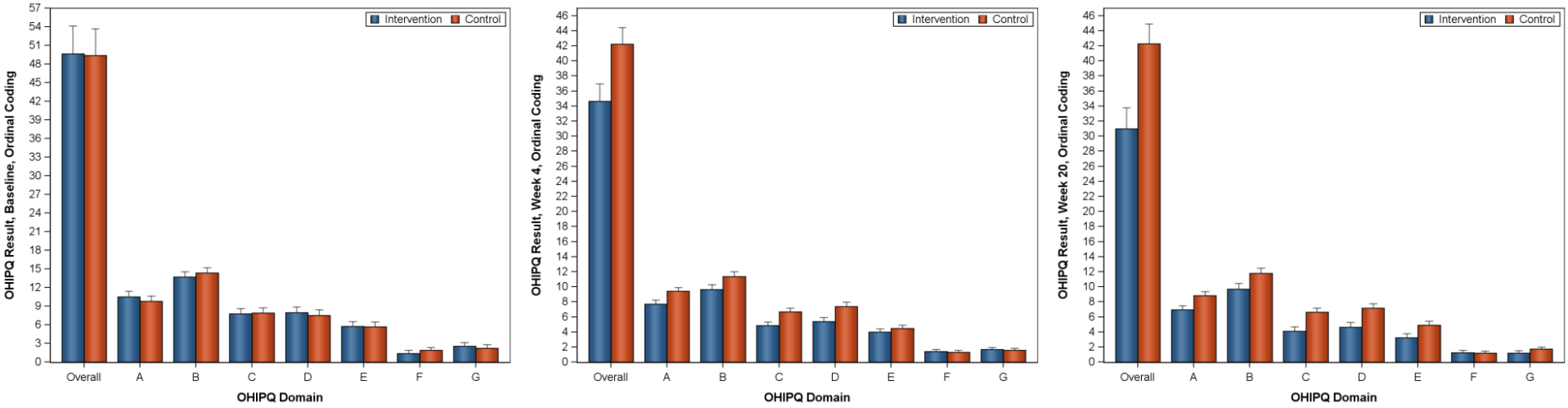


Figure 30. Clinical Trial 2. Bar Chart: Oral Health Impact (OHIP) scores for ordinal coding, MITT Subjects.
Domains: A=Functional Limitation, B=Physical Pain, C=Psychological Discomfort, D=Physical Disability, E=Psychological Disability, F=Social Disability, G=Handicap. Bars represent least square means. Vertical lines represent standard error of the LS means.

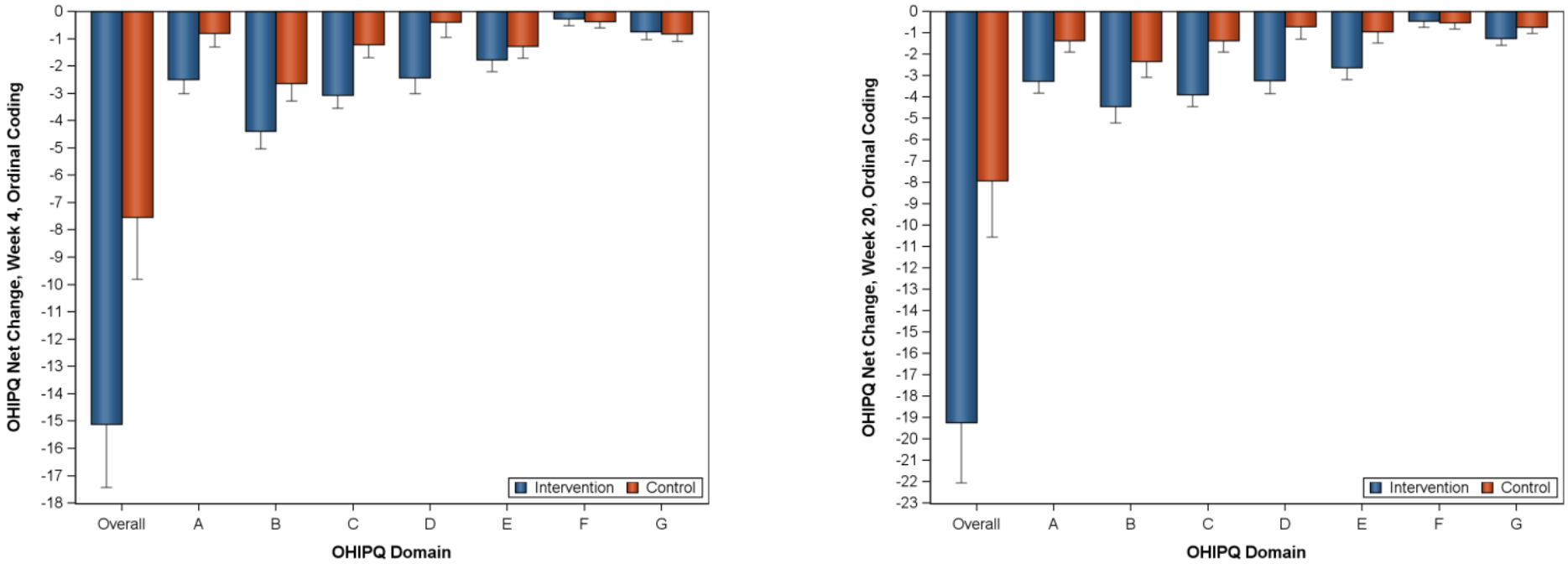


Figure 31. Clinical trial 2. Bar Chart: Oral Health Impact (OHIP) net change scores for ordinal coding MITT Subjects.
OHIP Domains: A=Functional Limitation, B=Physical Pain, C=Psychological Discomfort, D=Physical Disability, E=Psychological Disability, F=Social Disability, G=Handicap. Bars represent least square means. Vertical lines represent standard error of the LS means.

3.9.2 Visual Analogue Scale (VAS)

Descriptive statistics are presented in Table 43. The treatment groups were similar at baseline with mean VAS 3.34 for intervention group and 3.36 for control group. The median at baseline was 3.00 for intervention group and 2.90 for control indicating that the data were not skewed.

The relationship between baseline and post-baseline is presented by box plots (Figure 32) and reference plots (Figure 33). In these reference plots, scores below the reference line indicate increase at post-baseline compared to baseline, whilst values above the reference line indicate a decrease at post baseline. At week 4 and week 20, both groups' distributions shift with a reduction in VAS pain. The shift for the intervention group was slightly more than the control group.

Least squares (LS) means and statistical output are presented in Table 44. The overall LS mean VAS for pain reduction was 1.11 at week 4 and 1.62 at week 20 for the intervention group. The overall LS mean VAS for pain reduction was 0.44 at week 4 and 0.90 at week 20 for the control group. The LS mean treatment difference (Intervention – Control) and 95% CI was 0.67 (-0.04, 1.39) at week 4 and 0.72 (-0.06, 1.50) at week 20. The reduction in pain in the intervention group was more than in the control group, although the difference was not statistically significant (p=0.064, p=0.069) it was maintained at week 4 and week 20.

Visit	Outcome	Treatment	n	Mean (SD)	Median	Min, Max	95% CI
Baseline	Result	Intervention	39	3.34 (2.07)	3.00	0.00, 7.20	2.67, 4.01
		Control	43	3.36 (2.23)	2.90	0.00, 8.10	2.67, 4.05
Week 4	Result	Intervention	38	2.27 (1.66)	2.05	0.00, 7.20	1.73, 2.82
		Control	40	2.95 (2.06)	2.55	0.00, 7.60	2.29, 3.61
	Reduction from Baseline	Intervention	38	1.11 (1.80)	1.30	-2.20, 4.60	0.52, 1.70
		Control	40	0.44 (2.11)	0.20	-4.40, 5.80	-0.24, 1.11
Week 20	Result	Intervention	36	1.85 (1.72)	1.43	0.00, 6.80	1.27, 2.43
		Control	41	2.49 (2.04)	2.60	0.00, 9.20	1.85, 3.14
	Reduction from Baseline	Intervention	36	1.68 (2.07)	1.50	-2.30, 5.40	0.98, 2.38
		Control	41	0.84 (2.21)	0.40	-7.10, 5.90	0.14, 1.54

Table 43. Clinical Trial 2. Visual Analogue Scale (VAS) for pain. Descriptive summary, MITT Subjects. 10cm VAS scale responses were rounded to the nearest mm.

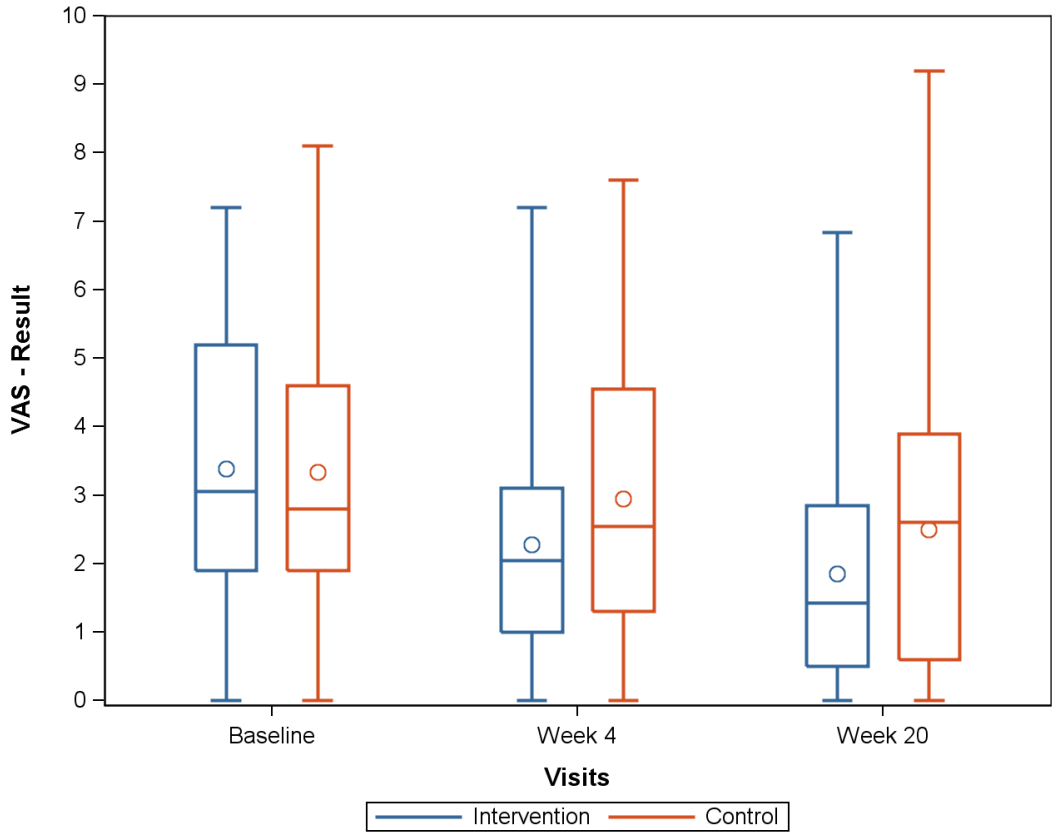
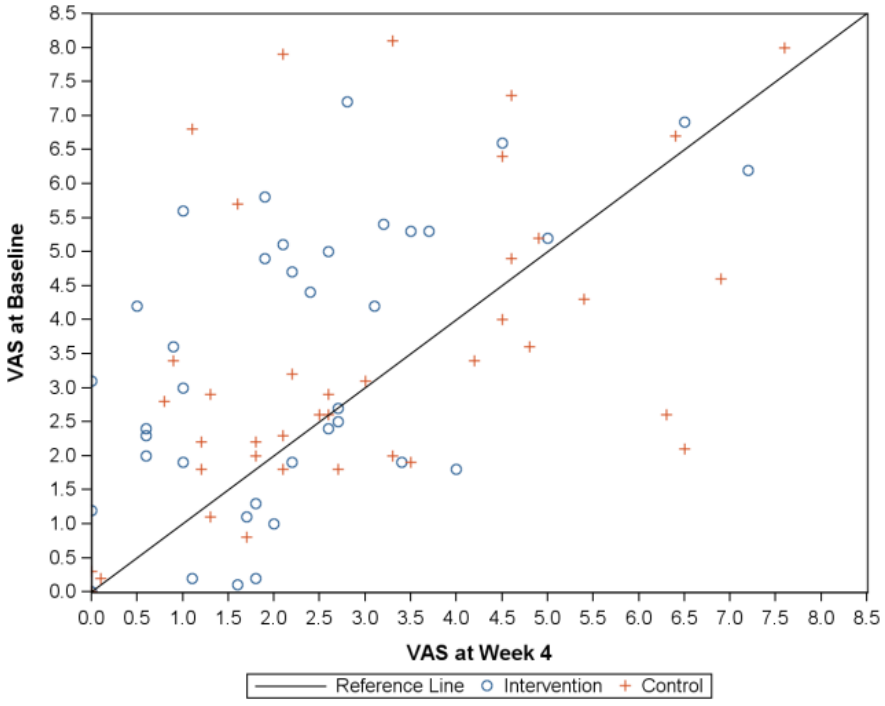


Figure 32. Clinical Trial 2. Box Plot of Visual Analogue Scale (cm) over time, MITT subjects.
At the 4- and 20-week follow-up, both groups show improvement in VAS score. The differences between the group means were however not statistically significant at follow-up (ANOVA p=0.9191 at baseline; p=0.0646 at week 4; p=0.0693 at week 20).



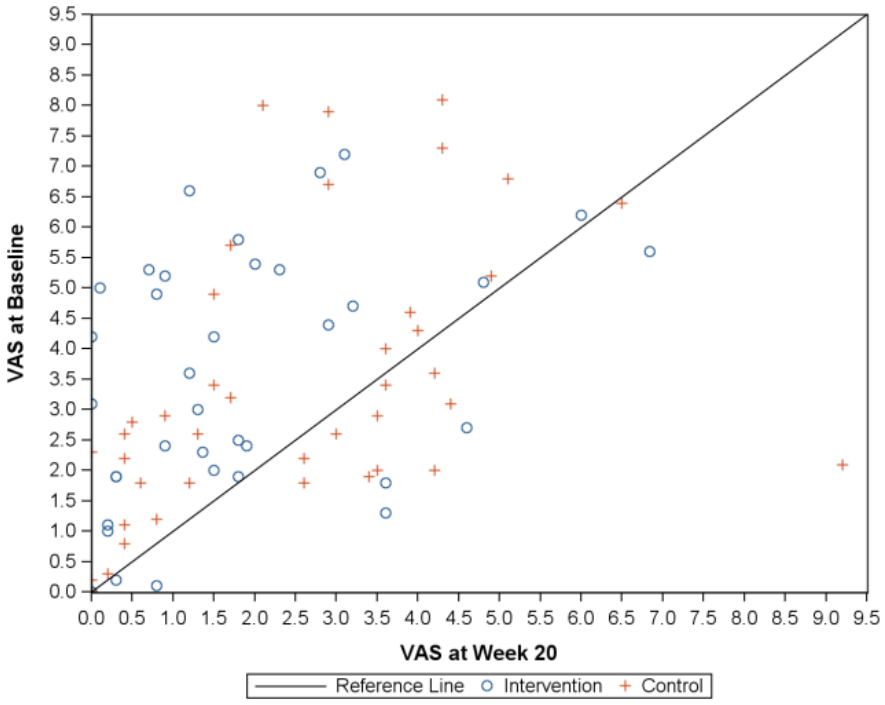


Figure 33. Clinical Trial 2. Reference Plot: Visual Analogue Scale (cm), MITT Subjects. Plots above the reference line indicate lower pain symptoms from baseline and those below the reference point indicate subjects with increases in pain.
There are general improvements in both intervention and control subjects. More Intervention subjects improve overall than control subjects

Visit	Outcome	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	3.38 (0.35)	3.33 (0.34)	0.05 (0.49)	0.9191
		95% CI	2.68, 4.09	2.66, 4.01	-0.93, 1.03	
Week 4	Result	n	38	41		
		LS Mean (SE)	2.27 (0.26)	2.95 (0.25)	-0.67 (0.36)	0.0646
		95% CI	1.76, 2.79	2.45, 3.45	-1.39, 0.04	
	Reduction from Baseline	n	38	40		
		LS Mean (SE)	1.11 (0.26)	0.44 (0.25)	0.67 (0.36)	0.0646
		95% CI	0.60, 1.62	-0.06, 0.94	-0.04, 1.39	
Week 20	Result	n	38	41		
		LS Mean (SE)	1.81 (0.29)	2.53 (0.27)	-0.72 (0.39)	0.0693
		95% CI	1.24, 2.38	2.00, 3.06	-1.50, 0.06	
	Reduction from Baseline	n	36	41		
		LS Mean (SE)	1.62 (0.29)	0.90 (0.27)	0.72 (0.39)	0.0693
		95% CI	1.05, 2.19	0.37, 1.43	-0.06, 1.50	

Table 44. Clinical Trial 2. Visual Analogue Scale (VAS) for pain. Least squares means, MITT subjects.
ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error. [a]P-value is based on a mixed model F-test (Ho: Both treatments equal). [b] Diff = Mean (SD) of the treatment difference (Intervention - Control).

3.9.3 Plaque Index (PI) (Silness and Löe, 1964)

The two groups were similar at baseline (p=0.6928) with mean PI 1.42 for the intervention group and 1.45 for the control group. Descriptive statistics are presented for PI in Table 45 and box plots for PI are presented in Figure 34. The relationship between baseline and post-baseline for PI is presented in Figure 35. In this reference plot scores below the reference line indicate an increase post-baseline compared to baseline, whilst values above the reference line indicate a decrease post-baseline.

At weeks 4 and 20, only the intervention group showed distribution shift. The overall mean PI was calculated as an arithmetic mean of all PI scores recorded at 6 sites per tooth as detailed in the methods.

The overall unadjusted PI reduction was 0.54 (36.32%) at week 4 and 0.57 (39.45%) at week 20 for the intervention group. The overall unadjusted PI reduction was -0.01 (-0.59%) at week 4 and -0.03 (-4.04%) at week 20 for the control group.

The overall least squares mean PI reductions are presented in Table 46 and were 0.53 (36.55%) at week 4 and 0.57 (39.56%) at week 20 for the intervention group. The overall LS mean PI reductions were 0.00 (0.81%) at week 4 and -0.03 (-4.13%) at week 20 for the control group. The LS mean treatment difference (Intervention – Control) and 95% CI reduction was 0.53 (0.42, 0.64) at week 4 and 0.60 (0.48, 0.73) at week 20. The difference in reduction in PI between intervention and control groups was statistically significant (p<0.0001) at both follow-up visits.

Visit	Outcome	Treatment	n	Mean (SD)	Median	Min, Max	95% CI
Baseline	Result	Intervention	39	1.42 (0.36)	1.35	0.70, 2.40	1.30, 1.54
		Control	43	1.45 (0.34)	1.41	0.85, 2.17	1.34, 1.55
Week 4	Result	Intervention	38	0.89 (0.33)	0.84	0.38, 2.04	0.78, 0.99
		Control	40	1.44 (0.34)	1.49	0.68, 2.05	1.33, 1.55
	Reduction from Baseline	Intervention	38	0.53 (0.33)	0.49	-0.44, 1.36	0.42, 0.63
		Control	40	0.01 (0.22)	0.03	-0.46, 0.49	-0.06, 0.08
	% Reduction from Baseline	Intervention	38	36 (21)	37	-50, 67	29, 43
		Control	40	-1 (18)	2	-53, 42	-6, 5
Week 20	Result	Intervention	36	0.86 (0.34)	0.88	0.22, 1.54	0.75, 0.98
		Control	41	1.47 (0.32)	1.42	0.86, 2.43	1.37, 1.57
	Reduction from Baseline	Intervention	36	0.57 (0.36)	0.56	-0.12, 1.48	0.45, 0.69
		Control	41	-0.03 (0.27)	-0.08	-0.49, 0.75	-0.11, 0.06
	% Reduction from Baseline	Intervention	36	39 (22)	42	-9, 86	32, 47
		Control	41	-4 (20)	-6	-58, 35	-10, 2

Table 45. Clinical Trial 2. Plaque Index (Silness and Löe, 1964), descriptive summary, MITT subjects.

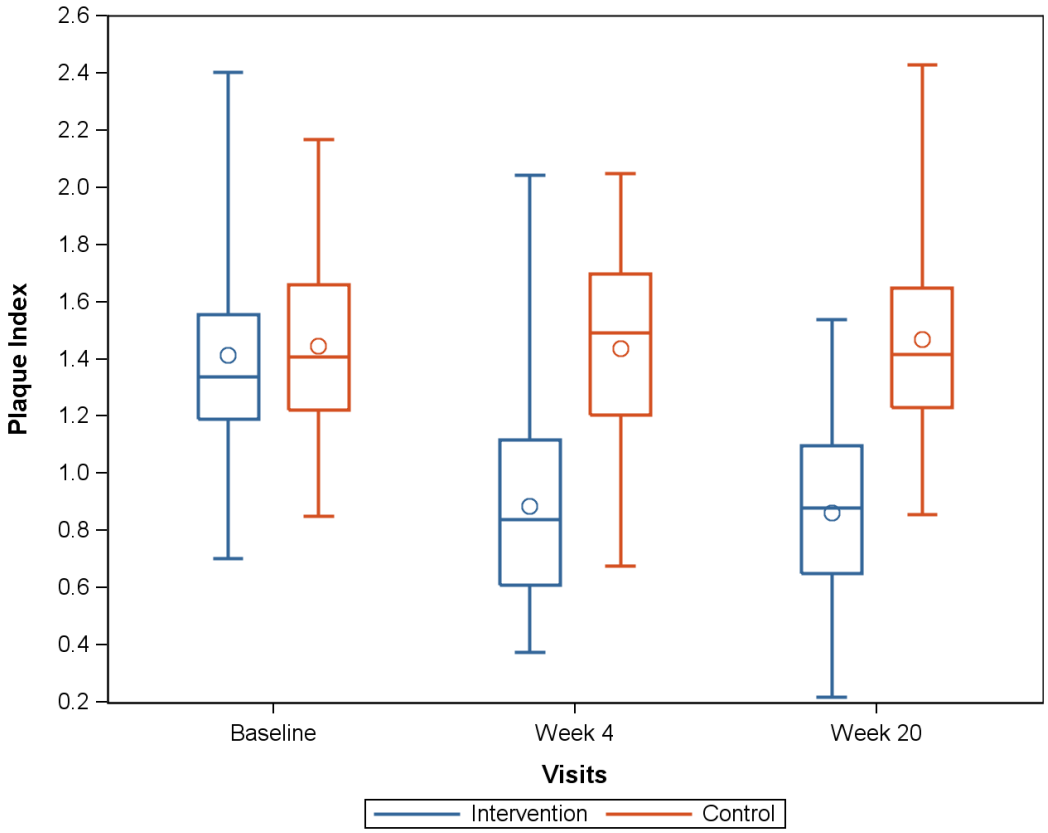
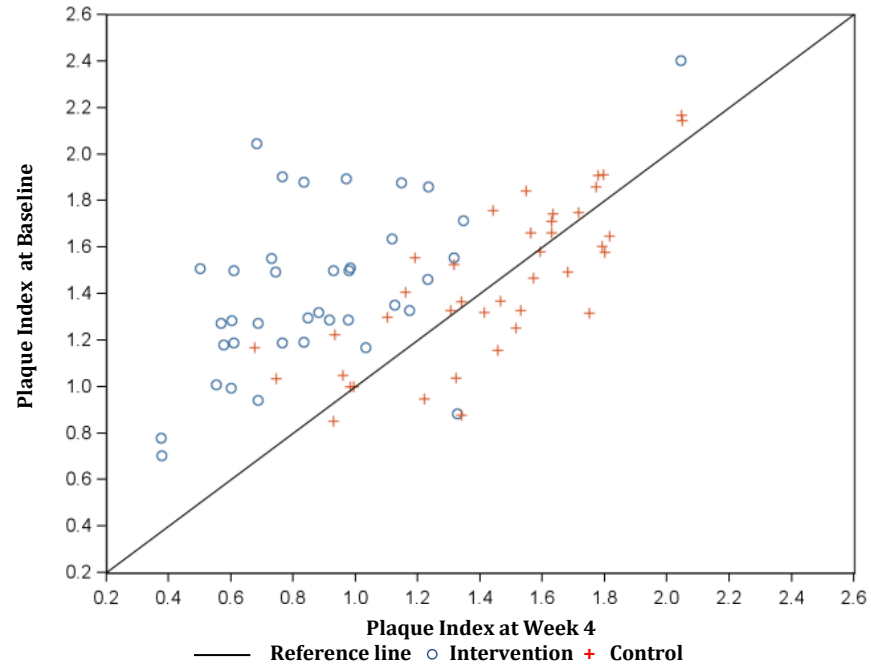


Figure 34. Clinical Trial 2. Box Plot: Plaque Index (Silness and Löe, 1964), MITT Subjects. At the 4- and 20-week follow-up, both groups show improvement in Plaque Index. The differences between the group means were statistically significant at follow-up (ANOVA p=0.6928 at baseline; p<0.0001 at week 4; p=<0.0001 at week 20).



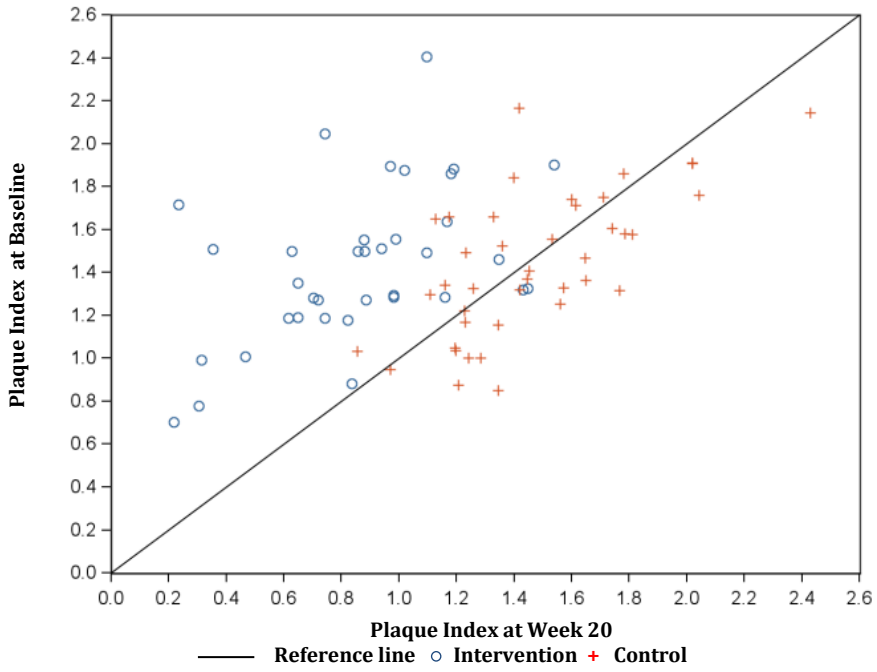


Figure 35. Clinical Trial 2. Reference Plot: Plaque Index (Silness and Löe, 1964), MITT Subjects.
Plots above the reference line indicate lower plaque indices from baseline and those below the reference point indicate subjects with increases in plaque.
Generally there is a higher concentration of intervention subjects with plots above the reference lines at weeks 4 and 20 indicating an improvement in plaque control.

Visit	Variable	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	1.41 (0.06)	1.44 (0.05)	-0.03 (0.08)	0.6928
		95% CI	1.30, 1.53	1.34, 1.55	-0.19, 0.13	
Week 4	Result	n	38	41		
		LS Mean (SE)	0.90 (0.04)	1.43 (0.04)	-0.53 (0.06)	<0.0001
		95% CI	0.82, 0.98	1.35, 1.51	-0.64, -0.42	
	Reduction from Baseline	n	38	40		
		LS Mean (SE)	0.53 (0.04)	0.00 (0.04)	0.53 (0.06)	<0.0001
		95% CI	0.45, 0.61	-0.07, 0.08	0.42, 0.64	
	% Reduction from Baseline	n	38	40		
		LS Mean (SE)	37 (3)	-1 (3)	37 (4)	<0.0001
		95% CI	30, 43	-7, 6	29, 46	
Week 20	Result	n	38	41		
		LS Mean (SE)	0.87 (0.05)	1.47 (0.04)	-0.60 (0.06)	<0.0001
		95% CI	0.77, 0.96	1.38, 1.55	-0.73,-0.48	
	Reduction from Baseline	n	36	41		
		LS Mean (SE)	0.57 (0.05)	-0.03 (0.04)	0.60 (0.06)	<0.0001
		95% CI	0.48, 0.67	-0.11, 0.06	0.48, 0.73	
	% Reduction from Baseline	n	36	41		
		LS Mean (SE)	40	-4 (3)	44 (5)	<0.0001
		95% CI	33, 46	-10, 2	35, 53	

Table 46. Clinical Trial 2. Plaque Index (Silness and Löe, 1964), overall least squares means, MITT subjects.
ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error.
[a]P-value is based on a mixed model F-test (Ho: Both treatments equal).
[b] Diff = Mean (SD) of the treatment difference (Intervention - Control).

3.9.4 Oro-mucosal disease score (Escudier *et al.*, 2007)

The individual components that comprise the Escudier oro-mucosal disease score (site, severity and activity) were assessed along with the weighted overall score that included the subjective assessment of pain. The scores from the VAS 10cm scale were used to complete the weighted score. Descriptive statistics for the individual components of the Oro-Mucosal Disease Scores are presented in Table 47 for site score, Table 48 for severity score and Table 49 for activity score. For the site, severity and activity scores, the medians at baseline were similar indicating that the data were not skewed. The mean baseline severity and activity scores were slightly higher in the intervention group at baseline.

The two groups had a similar distributions at baseline. At week 4 and week 20 both groups showed a distribution shift, and a reduction in site, severity and activity scores compared to baseline. The shift in intervention group was greater than the control. Box plots for change in site, severity and activity scores are presented in Figure 36. The relationships between baseline and post-baseline are presented in reference plot form in Figure 37. The plot scores below the reference line indicate an increase post-baseline compared to baseline, while scores above the reference line indicate a decrease post-baseline compared with baseline. Bar charts showing the reduction from baseline for the site, severity and activity scores are presented in Figure 38.

The LS means statistical output for oro-mucosal disease scores are presented in Table 50 (site), Table 51 (severity), Table 52 (activity) along with descriptive statistics and LS means for the weighted overall scores (Table 53). At baseline there were no statistical differences between the two groups ($p>0.05$). At follow up the LS mean reduction was statistically different between the two groups ($p<0.001$) for site, severity and activity scores. The LS mean treatment difference (intervention – control) and 95% CI in reduction in the oro-mucosal disease individual component scores were all statistically significant ($p<0.01$). Graphical representation of the overall weighted score from baseline is presented in Figure 39.

When combined with pain scores to give the weighted score there was no difference between the groups at baseline ($p=0.128$) but a statistically significant difference in weighted score at week 4 ($p=0.027$) and week 20 ($p=0.026$). The

overall reduction in weighted oro-mucosal disease score in the intervention group was more than the control group at weeks 4 and 20 ($p<0.001$).

Visit	Outcome	Treatment	n	Mean (SD)	Median	Min, Max	95% CI
Baseline	Result	Intervention	39	10.87 (2.52)	11.0	7.0, 17.0	10.06, 11.69
		Control	43	10.35 (2.25)	10.0	7.0, 15.0	9.66, 11.04
Week 4	Result	Intervention	38	9.45 (2.65)	9.0	5.0,16.0	8.58, 10.32
		Control	40	10.63 (2.20)	10.0	7.0, 15.0	9.92, 11.33
	Reduction from Baseline	Intervention	38	1.32 (1.74)	1.0	-3.0, 5.00	0.74, 1.89
		Control	40	-0.15 (1.31)	0.0	-3.0, 2.0	-0.57, 0.27
	% Reduction from Baseline	Intervention	38	12 (17)	13	-27, 45	6, 17
		Control	40	-2 (13)	0.0	-30, 22	-7, 2
Week 20	Result	Intervention	36	9.14 (2.55)	9.0	4.0, 15.0	8.27, 10.00
		Control	40	10.50 (2.06)	10.5	7.0, 14.0	9.84, 11.16
	Reduction from Baseline	Intervention	36	1.72 (1.77)	1.5	-2.0, 6.0	1.12, 2.32
		Control	40	-0.05 (1.71)	0.0	-7.0, 2.0	-0.60, 0.50
	% Reduction from Baseline	Intervention	36	15 (17)	12.5	-29, 60	9, 21
		Control	40	-2 (21)	0.0	-100, 22	-9, 4

Table 47. Clinical Trial 2. Escudier oro-mucosal disease score descriptive summary for the **site score**, MITT subjects.

Visit	Variable	Treatment	n	Mean (SD)	Median	Min, Max	95% CI
Baseline	Result	Intervention	39	14.92 (5.64)	13.0	6.0, 29.0	13.10, 16.75
		Control	43	12.86 (4.30)	13.0	3.0, 21.0	11.54, 14.18
Week 4	Result	Intervention	38	10.79 (5.04)	10.0	3.0, 22.0	9.13, 12.45
		Control	40	13.53 (4.46)	13.5	5.0, 22.0	12.10, 14.95
	Reduction from Baseline	Intervention	38	3.89 (4.07)	4.0	-4.0, 13.0	2.56, 5.23
		Control	40	-0.53 (3.37)	-1.0	-7.0, 8.0	-1.60, 0.55
	% Reduction from Baseline	Intervention	38	25 (28)	25	-50, 68	15, 34
		Control	40	-8 (27)	-7	-67, 38	-17, 1
Week 20	Result	Intervention	36	9.67 (5.17)	9.0	1.0, 20.0	7.92, 11.42
		Control	40	11.98 (3.69)	12.0	6.0, 19.0	10.79, 13.16
	Reduction from Baseline	Intervention	36	5.19 (4.47)	5.5	-4.0, 14.0	3.68, 6.71
		Control	40	1.03 (3.91)	0.0	-8.0, 12.0	-0.23, 2.28
	% Reduction from Baseline	Intervention	36	34 (30)	39	-33, 89	24, 44
		Control	40	-1 (50)	0	-267, 63	-17, 15

Table 48. Clinical Trial 2. Escudier oro-mucosal disease score descriptive summary for the **severity score**, MITT Subjects.

Visit	Variable	Treatment	n	Mean (SD)	Median	Min, Max	95% CI
Baseline	Result	Intervention	39	16.82 (7.06)	15.0	6.0, 35.0	14.53, 19.11
		Control	43	13.98 (5.25)	14.0	3.0, 27.0	12.36, 15.59
Week 4	Result	Intervention	38	11.66 (6.04)	10.0	4.0, 28.0	9.67, 13.64
		Control	40	15.03 (5.95)	15.0	5.0, 28.0	13.12, 16.93
	Reduction from Baseline	Intervention	38	4.87 (4.77)	4.5	-3.0, 17.0	3.30, 6.43
		Control	40	-0.88 (3.76)	-1.0	-9.0, 8.0	-2.08, 0.33
	% Reduction from Baseline	Intervention	38	27 (28)	28	-50, 69	18, 36
		Control	40	-10 (28)	-6	-67, 41	-19, -1
Week 20	Result	Intervention	36	10.31 (6.06)	8.0	1.0, 22.0	8.25, 12.36
		Control	40	12.75 (4.63)	12.0	6.0, 25.0	11.27, 14.23
	Reduction from Baseline	Intervention	36	6.36 (5.20)	7.0	-5.0, 15.0	4.60, 8.12
		Control	40	1.25 (4.44)	1.0	-8.0, 12.0	-0.17, 2.67
	% Reduction from Baseline	Intervention	36	37 (31)	44	-38, 89	27, 48
		Control	40	-0 (51)	7	-267, 63	-16, 16

Table 49. Clinical Trial 2. Escudier oro-mucosal disease score descriptive summary for the **activity score**, MITT Subjects.

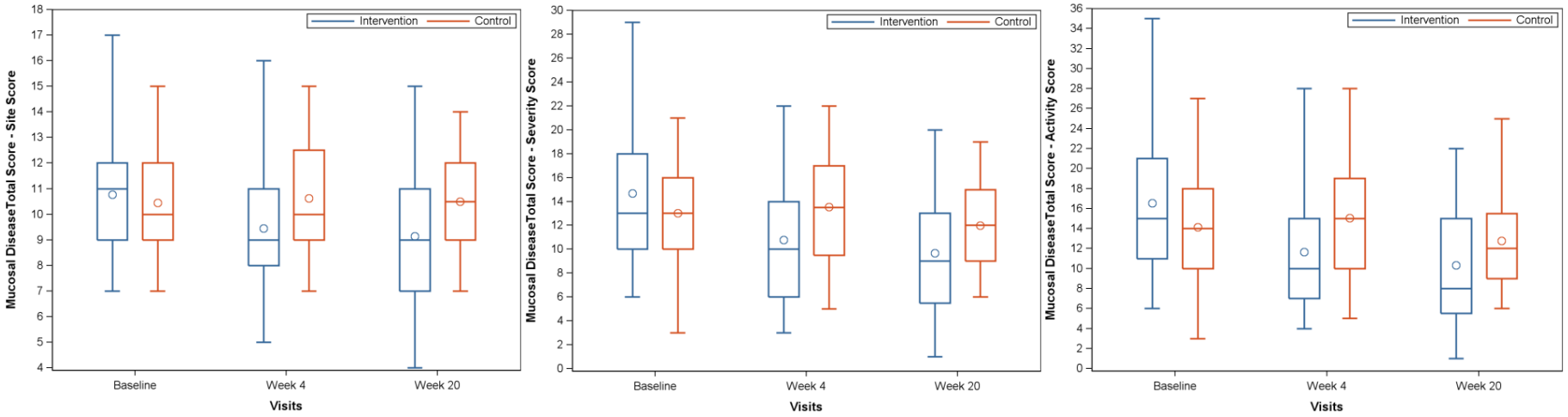


Figure 36. Clinical Trial 2. Box Plots: Escudier oro-mucosal disease scores for site, severity and activity over time.

The site score showed no significant difference between the means at baseline (ANOVA p=0.5409); at follow-up significant differences were observed between the means (ANOVA p<0.0001 at week 4, p<0.0001 at week 20).
The severity score showed no significant difference between the means at baseline (ANOVA p=0.1340); at follow up significant differences were observed between the means (ANOVA p<0.0001 at week 4, p<0.0001 at week 20).
The activity score showed small but not statistically significant difference at baseline (ANOVA p=0.086); at follow up significant differences were observed between the means (ANOVA p<0.0001 at week 4, p<0.0001 at week 20).

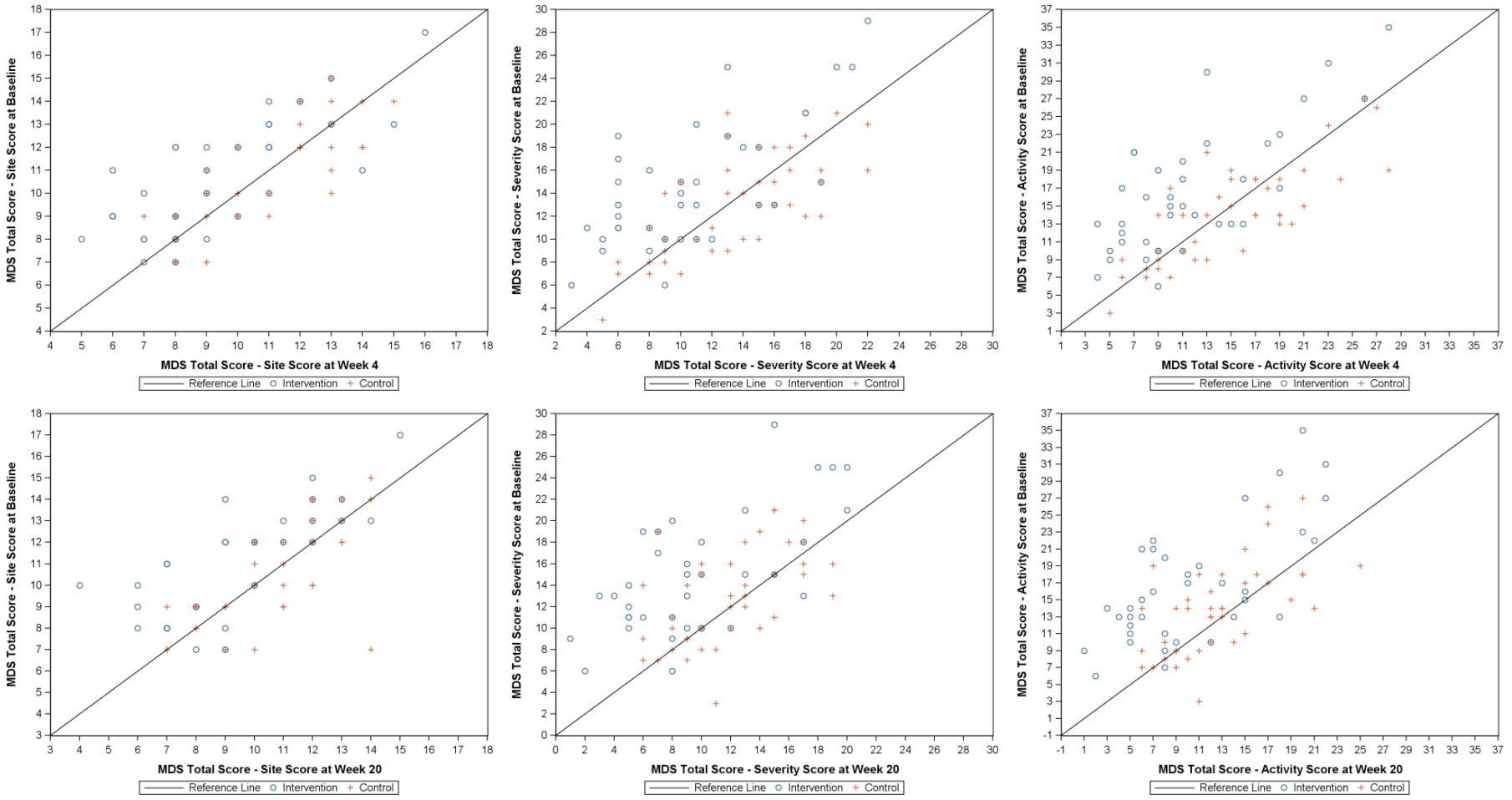


Figure 37. Clinical Trial 2. Reference plots: Escudier oro-mucosal disease score for site, severity, and activity over time, MITT Subjects.
The upper row of reference plots show plots at week-4 against baseline and the lower row at 20-weeks against baseline. Plots above the reference line indicate subjects with improvements from baseline whilst those below the reference line indicate subjects who deteriorated from baseline.

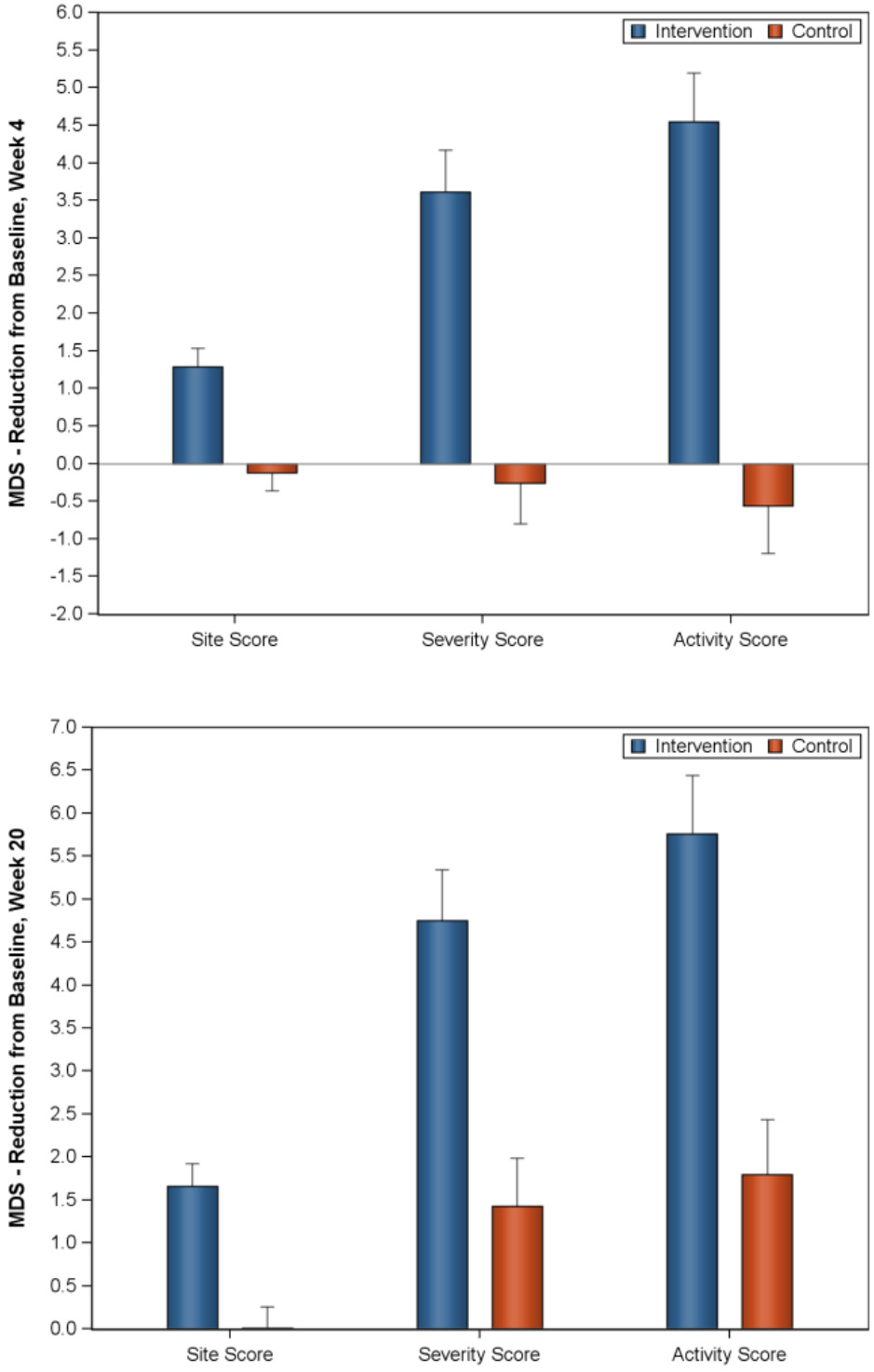


Figure 38. Clinical Trial 2. Bar chart: Escudier oro-mucosal disease site, severity and activity scores showing reduction from baseline, MITT subjects.
Bars represent least square means. Vertical lines represent standard error of the LS means.
At week 4, mean reductions in site, severity and activity score components were all significantly different between groups (ANOVA $p<0.0001$). At week 20, mean reductions in site severity and activity score components were all significantly different between groups (ANOVA $p<0.0001$ for site score, $p=0.0001$ for severity score; $p<0.0001$ for activity score).

Visit	Variable	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	n	38	41		
		LS Mean (SE)	10.76 (0.38)	10.44 (0.37)	0.32 (0.53)	0.5409
		95% CI	10.01, 11.52	9.71, 11.17	-0.73, 1.38	
Week 4	Result	n	38	41		
		LS Mean (SE)	9.33 (0.24)	10.74 (0.24)	-1.41 (0.34)	<0.0001
		95% CI	8.85, 9.81	10.27, 11.21	-2.09, -0.74	
	Reduction from Baseline	n	38	40		
		LS Mean (SE)	1.29 (0.24)	-0.12 (0.24)	1.41 (0.34)	<0.0001
		95% CI	0.81, 1.77	-0.59, 0.34	0.74, 2.09	
	% Reduction from Baseline	n	38	40		
		LS Mean (SE)	12 (2)	-2 (2)	14 (3)	<0.0001
		95% CI	7, 16	-7, 2	7, 20	
Week 20	Result	n	38	41		
		LS Mean (SE)	8.99 (0.27)	10.64 (0.25)	-1.65 (0.37)	<0.0001
		95% CI	8.45, 9.52	10.13, 11.14	-2.39, -0.92	
	Reduction from Baseline	n	36	40		
		LS Mean (SE)	1.66 (0.27)	0.01 (0.25)	1.65 (0.37)	<0.0001
		95% CI	1.13, 2.19	-0.50, 0.51	0.92, 2.39	
	% Reduction from Baseline	n	36	40		
		LS Mean (SE)	15 (3)	-2 (3)	17 (4)	0.0002
		95% CI	9, 21	-8, 4	8, 25	

Table 50. Clinical Trial 2. Escudier oro-mucosal disease **site score**, least squares means, MITT subjects
ANOVA Model for baseline: Result=Treatment + error.
ANOVA Model for post-baseline: Result=Treatment + Baseline + Error.
[a]P-value is based on a mixed model F-test (Ho: Both treatments equal).
[b] Difference = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Variable	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	N	38	41		
		LS Mean (SE)	14.68 (0.80)	13.00 (0.77)	1.68 (1.11)	0.1340
		95% CI	13.09, 16.28	11.46, 14.54	-0.53, 3.90	
Week 4	Result	N	38	41		
		LS Mean (SE)	10.21 (0.55)	14.08 (0.54)	-3.87 (0.78)	<0.0001
		95% CI	9.10, 11.31	13.01, 15.15	-5.42, -2.33	
	Reduction from Baseline	N	38	40		
		LS Mean (SE)	3.61 (0.55)	-0.26 (0.54)	3.87 (0.78)	<0.0001
		95% CI	2.51, 4.72	-1.33, 0.81	2.33, 5.42	
	% Reduction from Baseline	N	38	40		
		LS Mean (SE)	23 (4)	-7 (4)	30(6)	<0.0001
		95% CI	15, 32	-15, 2	18, 42	
Week 20	Result	N	38	41		
		LS Mean (SE)	9.13 (0.60)	12.46 (0.56)	-3.32 (0.83)	0.0001
		95% CI	7.95, 10.32	11.33, 13.58	-4.97, -1.68	
	Reduction from Baseline	N	36	40		
		LS Mean (SE)	4.75 (0.60)	1.43 (0.56)	3.32 (0.83)	0.0001
		95% CI	3.56, 5.94	0.30, 2.55	1.68, 4.97	
	% Reduction from Baseline	N	36	40		
		LS Mean (SE)	31 (7)	1 (6)	30 (9)	0.0021
		95% CI	18, 44	-11, 14	11, 48	

Table 51. Clinical Trial 2. Escudier oro-mucosal disease **severity score**, least squares means, MITT subjects.
ANOVA Model for baseline: Result=Treatment + error.
ANOVA Model for post-baseline: Result=Treatment + Baseline + Error.
[a]P-value is based on a mixed model F-test (Ho: Both treatments equal).
[b] Difference = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Variable	Statistic	Treatment		Difference[b]	P-value[a]
			Intervention	Control		
Baseline	Result	N	38	41		
		LS Mean (SE)	16.53 (1.00)	14.12 (0.96)	2.40 (1.38)	0.0861
		95% CI	14.54, 18.51	12.21, 16.03	-0.35, 5.16	
Week 4	Result	N	38	41		
		LS Mean (SE)	10.77 (0.65)	15.87 (0.63)	-5.10 (0.92)	<0.0001
		95% CI	9.47, 12.06	14.61, 17.13	-6.93, -3.28	
	Reduction from Baseline	N	38	40		
		LS Mean (SE)	4.54 (0.65)	-0.56 (0.63)	5.10 (0.92)	<0.0001
		95% CI	3.24, 5.84	-1.83, 0.70	3.28, 6.93	
	% Reduction from Baseline	N	38	40		
		LS Mean (SE)	26 (4)	-8 (4)	34 (6)	<0.0001
		95% CI	17, 35	-17, 0	22, 47	
Week 20	Result	N	38	41		
		LS Mean (SE)	9.50 (0.68)	13.47 (0.65)	-3.97 (0.95)	<0.0001
		95% CI	8.14, 10.86	12.18, 14.76	-5.86, -2.07	
	Reduction from Baseline	N	36	40		
		LS Mean (SE)	5.76 (0.68)	1.79 (0.65)	3.97 (0.95)	<0.0001
		95% CI	4.40, 7.12	0.50, 3.08	2.07, 5.86	
	% Reduction from Baseline	N	36	40		
		LS Mean (SE)	35 (7)	2 (6)	32 (10)	0.0012
		95% CI	21, 48	-11, 15	13, 51	

Table 52. Clinical Trial 2. Escudier oro-mucosal disease **activity score**, least squares means, MITT subjects.
ANOVA Model for baseline: Result=Treatment + error.
ANOVA Model for post-baseline: Result=Treatment + Baseline + Error.
[a]P-value is based on a mixed model F-test (Ho: Both treatments equal).
[b] Difference = Mean (SD) of the treatment difference (Intervention - Control).

Visit	Outcome	Treatment	n	Mean (SD)	Median	Min, Max	95% CI	Mean difference (SD)[b]	P-value[a]
Baseline	Result	Intervention	39	31.12 (9.62)	30.90	16.30, 57.80	27.87, 34.37	3.14 (-0.93, 7.22)	0.128
		Control	43	27.97 (8.07)	28.00	14.00, 46.90	25.28, 30.52		
Week 4	Result	Intervention	38	23.93 (8.53)	20.85	12.50, 45.90	21.05, 26.81	-4.53 (-8.53, -0.53)	0.027
		Control	40	28.46 (8.82)	28.30	14.00, 49.40	25.60, 31.32		
	Reduction from Baseline	Intervention	38	7.18 (6.24)	6.95	-3.50, 22.00	5.07, 9.29	7.67 (5.05, 10.31)	<0.001
		Control	40	-0.49 (5.18)	-0.70	-12.40, 11.60	-2.17, 3.36		
	% Reduction from Baseline	Intervention	38	22 (18)	22	-19, 50	16, 28	24.46 (16.32, 32.62)	<0.001
		Control	40	-3 (17)	-4	-35, 31	-8, 3		
Week 20	Result	Intervention	36	21.40 (8.87)	18.70	5.00, 37.60	18.40, 24.40	-4.25 (-7.97, -0.52)	0.026
		Control	40	25.65 (7.31)	25.40	14.50, 47.20	23.28, 28.02		
	Reduction from Baseline	Intervention	36	9.71 (6.42)	9.71	-4.50, 21.00	7.53, 11.89	7.39 (4.49, 10.30)	<0.001
		Control	40	2.32 (6.18)	2.40	-12.10, 17.80	0.32, 4.32		
	% Reduction from Baseline	Intervention	36	31 (20)	30	-19, 78	24, 38	25.40 (15.56, 35.24)	<0.001
		Control	40	6 (22)	9	-80, 46	-2, 13		

Table 53. Clinical Trial 2. Escudier oro-mucosal disease index **weighted scores** (site, activity and pain), least squares mean treatment difference, MITT subjects.
ANOVA Model for baseline: Result=Treatment + error. ANOVA Model for post-baseline: Result=Treatment + Baseline + Error.
[a]P-value is based on a mixed model F-test (Ho: Both treatments equal).
[b] Difference = Mean (SD) of the treatment difference (Intervention - Control).

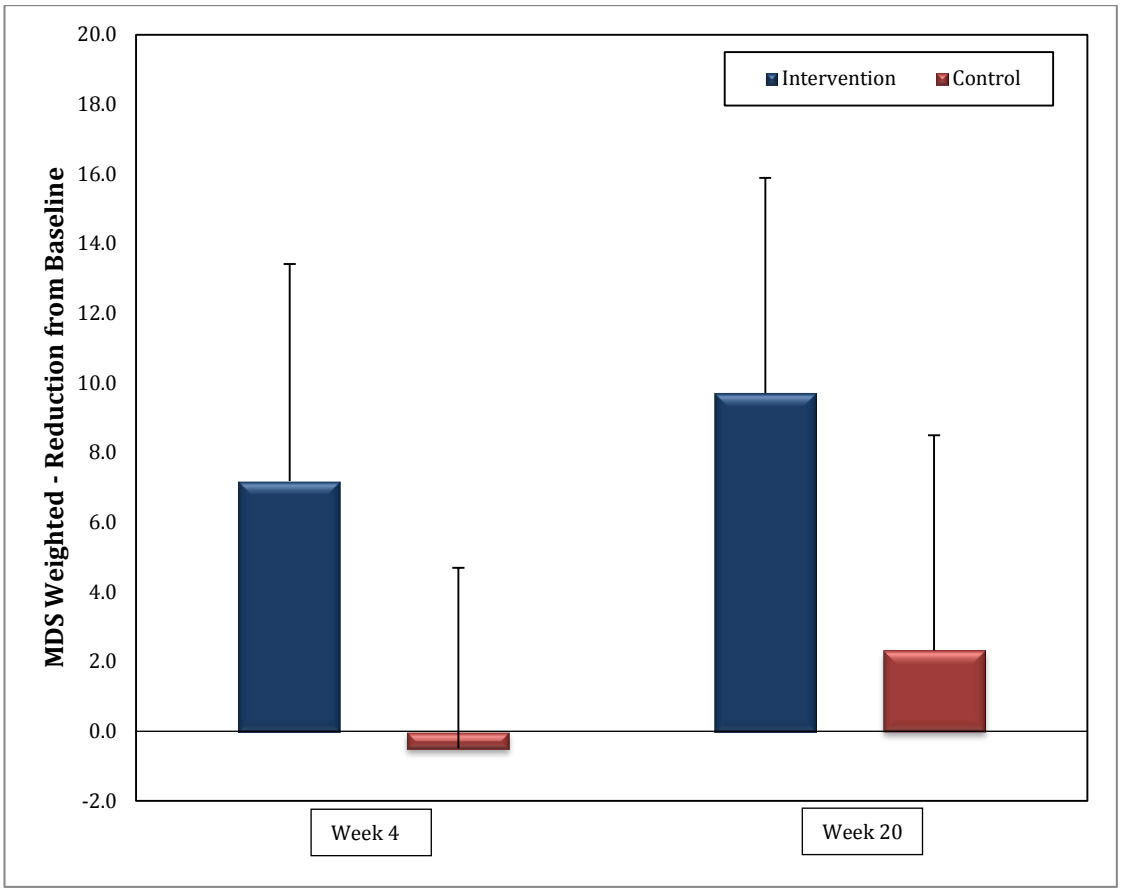


Figure 39. Clinical Trial 2. Bar chart: Escudier oro-mucosal disease index **weighted scores** reduction from baseline.
These represent the least squares mean sum of the site score, activity score and pain scores. Vertical lines represent standard deviation of the least square means. There were significant differences between groups at week 4 and week 20 (ANOVA $p<0.001$).

3.9.5 Biomarkers

Samples of saliva and GCF were obtained from a subset of the population (n=12) at baseline and week 4 to determine the local levels of inflammatory biomarkers. Both GCF and saliva were analysed as previously described (Chapter 2) for the following biomarkers: IL-1β, IL-2, MIP-1α, MIP-1β, RANTES, MMP-1, MMP-3, MMP-8, MMP-9 MMP-13. Descriptive statistics, and analysis of least squares means using analysis of variance are presented for the GCF samples in Table 54 for cytokines and Table 55 for MMPs. The data for saliva samples are presented in Table 56 for cytokines and Table 57 for MMPs.

Levels of all inflammatory biomarkers in both groups were similar at baseline in saliva ($p>0.05$) and GCF samples ($p>0.05$) although different levels of concentrations were identified in each fluid. Generally, higher concentrations of inflammatory mediators were present in GCF in comparison with saliva.

In the GCF samples, levels of MMP-1 and MMP-13 were detected below the minimum concentration threshold to have confidence in the result; in the saliva samples levels of IL-2, RANTES, MMP-1, MMP-13 were also not detected in high enough concentrations. The difference in RANTES in particular was at undetectable levels in the saliva samples yet found in comparatively high concentrations in GCF.

The mean biomarker concentrations were similar at the 4-week follow up for all observed biomarkers and no significant differences were observed following treatment ($p>0.05$).

Biomarker	Visit	Variable	Treatment	n	Mean (SD)[c]	Median	Min, Max	95% CI	Mean difference (SD)[b]	P-value[a]
IL-1β	Baseline	Result	Intervention	4	10.80 (0.39)	10.70	10.46, 11.34	10.17, 11.43	-0.55 (0.42)	0.2220
			Control	7	11.35 (0.77)	11.25	10.44, 12.90	10.64, 12.07		
	Week 4	Result	Intervention	4	11.44 (0.69)	11.35	10.75, 12.32	10.34, 12.54	-0.03 (0.48)	0.9443
			Control	7	11.53 (0.66)	11.31	10.45, 12.29	10.92, 12.14		
		Change from Baseline	Intervention	4	0.64 (0.75)	0.29	0.23, 1.76	-0.55, 1.83	-0.03 (0.48)	0.9443
			Control	7	0.18 (0.97)	0.07	-1.06, 1.84	-0.72, 1.07		
IL-2	Baseline	Result	Intervention	4	1.22 (2.44)	0.00	0.00, 4.88	-2.66, 5.11	0.50 (1.32)	0.7151
			Control	7	0.72 (1.91)	0.00	0.00, 5.07	-1.05, 2.49		
	Week 4	Result	Intervention	4	0.00 (0.00)	0.00	0.00, 0.00	N/a	-0.63 (0.97)	0.5325
			Control	7	0.67 (1.78)	0.00	0.00, 4.70	-0.97, 2.32		
		Change from Baseline	Intervention	4	-1.22 (2.44)	0.00	-4.88, 0.00	-5.11, 2.66	-0.63 (0.97)	0.5325
			Control	7	-0.05 (2.82)	0.00	-5.07, 4.70	-2.66, 2.56		
MIP-1α	Baseline	Result	Intervention	4	8.60 (0.99)	9.07	7.12, 9.15	7.03, 10.18	-0.09 (0.65)	0.8876
			Control	7	8.70 (1.05)	8.74	7.48, 10.23	7.72, 9.67		
	Week 4	Result	Intervention	4	9.48 (1.30)	10.04	7.55, 10.29	7.41, 11.55	0.34 (0.38)	0.3871
			Control	7	9.20 (0.65)	9.23	8.21, 10.25	8.60, 9.80		
		Change from Baseline	Intervention	4	0.88 (0.38)	0.91	0.43, 1.26	0.28, 1.47	0.34 (0.38)	0.3871
			Control	7	0.51 (0.74)	0.73	-0.81, 1.50	-0.18, 1.19		
MIP-1β	Baseline	Result	Intervention	4	6.64 (3.52)	8.20	1.41, 8.75	1.05, 12.23	-1.38 (1.42)	0.3581
			Control	7	8.02 (1.23)	7.76	6.08, 9.91	6.87, 9.16		
	Week 4	Result	Intervention	4	8.67 (1.43)	9.34	6.54, 9.48	6.40, 10.94	2.41 (1.61)	0.1746
			Control	7	7.33 (3.41)	8.41	0.00, 10.31	4.18, 10.48		
		Change from Baseline	Intervention	4	2.03 (2.10)	1.13	0.73, 5.13	-1.31, 5.37	2.41 (1.61)	0.1746
			Control	7	-0.69 (2.49)	0.20	-6.08, 1.35	-2.99, 1.62		

Biomarker	Visit	Variable	Treatment	n	Mean (SD)[c]	Median	Min, Max	95% CI	Mean difference (SD)[b]	P-value[a]
RANTES	Baseline	Result	Intervention	4	5.64 (4.05)	6.60	0.00, 9.38	-0.79, 12.08	0.48 (2.39)	0.8459
			Control	7	5.17 (3.68)	6.17	0.00, 9.15	1.76, 8.57		
	Week 4	Result	Intervention	4	7.67 (1.26)	7.88	6.12, 8.82	5.67, 9.68	0.22 (1.02)	0.8345
			Control	7	7.49 (1.69)	7.55	4.45, 9.26	5.93, 9.06		
		Change from Baseline	Intervention	4	2.03 (3.50)	0.75	-0.55, 7.18	-3.54, 7.60	0.22 (1.02)	0.8345
			Control	7	2.33 (4.77)	0.45	-3.05, 9.26	-2.08, 6.74		

Table 54. Clinical Trial 2. Descriptive summary: Biomarker GCF samples cytokine panel, log scale, MITT subjects.
Biomarkers concentration is calculated as the average of repeated measurements. Log transformation is applied to the average concentration. Change from baseline is calculated from the log-transformed concentration.
ANOVA Model for baseline: Result=Treatment + error.
ANOVA Model for post-baseline: Result=Treatment + Baseline + Error.
[a]P-value F-test (Ho: Both treatments equal).
[b] Difference = Mean (SD) of the treatment difference (Intervention - Control).
[c] Mean biomarker concentration in pg/ml.

Biomarker	Visit	Variable	Treatment	n	Mean (SD)[c]	Median	Min, Max	95% CI	Mean difference (SD)[b]	P-value[a]
MMP-1	Baseline	Result	Intervention	4	0.00 (0.00)	0.00	0.00, 0.00	N/a	-1.28 (1.73)	0.4790
			Control	7	1.28 (3.38)	0.00	0.00, 8.95	-1.85, 4.41		
	Week 4	Result	Intervention	4	0.00 (0.00)	0.00	0.00, 0.00	N/a	-1.14 (1.42)	0.4468
			Control	7	2.08 (3.56)	0.00	0.00, 7.72	-1.21, 5.37		
		Change from Baseline	Intervention	4	0.00 (0.00)	0.00	0.00, 0.00	N/a	-1.14 (1.42)	0.4468
			Control	7	0.80 (2.70)	0.00	-1.23, 6.83	-1.69, 3.29		
MMP-3	Baseline	Result	Intervention	4	4.83 (5.59)	4.66	0.00, 10.01	-4.06, 13.72	1.78 (3.35)	0.6092
			Control	7	3.05 (5.23)	0.00	0.00, 11.37	-1.78, 7.89		
	Week 4	Result	Intervention	4	2.50 (5.00)	0.00	0.00, 9.99	-5.45, 10.45	-0.36 (3.28)	0.9145
			Control	7	2.80 (4.80)	0.00	0.00, 10.26	-1.63, 7.24		
		Change from Baseline	Intervention	4	-2.33 (9.40)	-4.66	-10.01, 9.99	-17.29, 12.63	-0.36 (3.28)	0.9145
			Control	7	-0.25 (5.61)	0.00	-19.38	-5.44, 4.94		
MMP-8	Baseline	Result	Intervention	4	16.14 (0.26)	16.09	15.88, 16.50	15.72, 16.56	-0.77 (0.31)	0.0334
			Control	7	16.90 (0.57)	16.73	16.29, 17.78	16.38, 17.43		
	Week 4	Result	Intervention	4	16.49 (0.46)	16.59	15.89, 16.87	15.75, 17.22	-0.23 (0.30)	0.4789
			Control	7	16.90 (0.32)	16.93	16.35, 17.29	16.61, 17.20		
		Change from Baseline	Intervention	4	0.35 (0.29)	0.32	0.02, 0.73	-0.12, 0.81	-0.23 (0.30)	0.4789
			Control	7	-0.00 (0.58)	0.08	-1.71	-0.54, 0.54		
MMP-9	Baseline	Result	Intervention	4	16.89 (0.19)	16.89	16.69, 17.08	16.59, 17.19	-0.61 (0.36)	0.1222
			Control	7	17.50 (0.69)	17.80	16.49, 18.23	16.87, 18.13		
	Week 4	Result	Intervention	4	17.25 (0.37)	17.30	16.76, 17.64	16.66, 17.83	-0.11 (0.30)	0.7197
			Control	7	17.51 (0.44)	17.30	17.05, 18.20	17.11, 17.91		
		Change from Baseline	Intervention	4	0.36 (0.28)	0.41	-0.62	-0.08, 0.80	-0.11 (0.30)	0.7197
			Control	7	0.01 (0.68)	-0.04	-1.90	-0.62, 0.64		

Biomarker	Visit	Variable	Treatment	n	Mean (SD)[c]	Median	Min, Max	95% CI	Mean difference (SD)[b]	P-value[a]
MMP-13	Baseline	Result	Intervention	4	0.00 (0.00)	0.00	0.00, 0.00	N/a	0.00	N/a
			Control	7	0.00 (0.00)	0.00	0.00, 0.00	N/a		
	Week 4	Result	Intervention	4	0.00 (0.00)	0.00	0.00, 0.00	N/a	1.39 (1.88)	0.4790
			Control	7	1.39 (3.67)	0.00	0.00, 9.72	-2.01, 4.79		
		Change from Baseline	Intervention	4	0.00 (0.00)	0.00	0.00, 0.00	N/a	1.39 (1.88)	0.4790
			Control	7	1.39 (3.67)	0.00	0.00, 9.72	-2.01, 4.79		

Table 55. Clinical Trial 2. Descriptive summary: Biomarker GCF samples – MMP panel, log scale, MITT subjects.
Biomarkers concentration is calculated as the average of repeated measurement. Log transformation is applied to the average concentration. Change from baseline is calculated from the log-transformed concentration.
ANOVA Model for baseline: Result=Treatment + error.
ANOVA Model for post-baseline: Result=Treatment + Baseline + Error.
[a]P-value F-test (Ho: Both treatments equal).
[b] Difference = Mean (SD) of the treatment difference (Intervention - Control).
[c] Mean biomarker concentration in pg/ml.

Biomarker	Visit	Variable	Treatment	n	Mean (SD)[c]	Median	Min, Max	95% CI	Mean difference (SD)[b]	P-value[a]
IL-1β	Baseline	Result	Intervention	4	5.60 (1.10)	5.32	4.67, 7.09	3.84, 7.36	-0.88 (0.57)	0.1597
			Control	7	6.48 (0.80)	6.60	5.14, 7.74	5.74, 7.22		
	Week 4	Result	Intervention	4	6.10 (0.85)	6.11	5.09, 7.08	4.75, 7.45	0.18 (0.41)	0.6706
			Control	7	6.66 (1.00)	6.60	5.47, 8.47	5.74, 7.59		
		Change from Baseline	Intervention	4	0.50 (0.40)	0.53	-0.01, 0.95	-0.14, 1.14	0.18 (0.41)	0.6706
			Control	7	0.18 (0.63)	0.01	-0.47, 1.26	-0.40, 0.77		
IL-2	Baseline	Result	Intervention	4	0.50 (0.14)	0.45	0.40, 0.69	0.28, 0.72	0.07 (0.10)	0.4967
			Control	7	0.42 (0.18)	0.40	0.15, 0.64	0.26, 0.58		
	Week 4	Result	Intervention	4	0.59 (0.08)	0.58	0.50, 0.69	0.46, 0.71	0.02 (0.07)	0.8048
			Control	7	0.53 (0.16)	0.55	0.28, 0.77	0.38, 0.67		
		Change from Baseline	Intervention	4	0.09 (0.17)	0.08	-0.09, 0.29	-0.18, 0.36	0.02 (0.07)	0.8048
			Control	7	0.10 (0.10)	0.13	-0.1, 0.2	0.01, 0.20		
MIP-1α	Baseline	Result	Intervention	4	4.56 (0.47)	4.50	4.16, 5.10	3.81, 5.32	0.56 (0.32)	0.0268
			Control	7	4.01 (0.24)	4.03	3.53, 4.30	3.78, 4.23		
	Week 4	Result	Intervention	4	4.60 (0.57)	4.45	4.16, 5.35	3.70, 5.50	0.15 (0.32)	0.6541
			Control	7	4.19 (0.26)	4.16	3.97, 4.73	3.95, 4.44		
		Change from Baseline	Intervention	4	0.04 (0.15)	0.00	-0.1, 0.25	-0.20, 0.28	0.15 (0.32)	0.6541
			Control	7	0.19 (0.48)	0.10	-0.33, 1.2	-0.26, 0.63		

Biomarker	Visit	Variable	Treatment	n	Mean (SD)[c]	Median	Min, Max	95% CI	Mean difference (SD)[b]	P-value[a]
MIP-1β	Baseline	Result	Intervention	4	1.95 (1.67)	2.20	0.00, 3.42	-0.71, 4.62	0.75 (0.85)	0.4035
			Control	7	1.21 (1.17)	0.71	0.00, 3.62	0.13, 2.29		
	Week 4	Result	Intervention	4	2.18 (1.80)	2.18	0.00, 4.36	-0.69, 5.05	0.57 (0.93)	0.5580
			Control	7	1.29 (1.25)	1.51	0.00, 3.42	0.13, 2.45		
		Change from Baseline	Intervention	4	0.23 (0.87)	0.36	-0.89, 1.09	-1.16, 1.62	0.57 (0.93)	0.5580
			Control	7	0.09 (1.79)	0.67	-3.62, 1.81	-1.57, 1.74		
RANTES	Baseline	Result	Intervention	4	0.00 (0.00)	0.00	0.00, 0.00	N/a	-0.23 (0.31)	0.4790
			Control	7	0.23 (0.60)	0.00	0.00, 1.59	-0.33, 0.78		
	Week 4	Result	Intervention	4	0.70 (1.40)	0.00	0.00, 2.79	-1.52, 2.92	-0.70 (0.55)	0.2415
			Control	7	0.00 (0.00)	0.00	0.00, 0.00	N/a		
		Change from Baseline	Intervention	4	0.70 (1.40)	0.00	0.00, 2.79	-1.52, 2.92	-0.70 (0.55)	0.2415
			Control	7	-0.23 (0.60)	0.00	-1.59, 0.00	-0.78, 0.33		

Table 56. Clinical Trial 2. Descriptive summary: Biomarker saliva samples cytokine panel, log scale, MITT subjects.
Note: Biomarkers concentration is calculated as the average of repeated measurement. Log transformation is applied to the average concentration. Change from baseline is calculated from the log-transformed concentration.
ANOVA Model for baseline: Result=Treatment + error.
ANOVA Model for post-baseline: Result=Treatment + Baseline + Error.
[a]P-value F-test (Ho: Both treatments equal).
[b] Difference = Mean (SD) of the treatment difference (Intervention - Control).
[c] Mean biomarker concentration in pg/ml.

Biomarker	Visit	Variable	Treatment	n	Mean (SD)[c]	Median	Min, Max	95% CI	Mean difference (SD)[b]	P-value[a]
MMP-1	Baseline	Result	Intervention	4	2.77 (3.22)	2.54	0.00, 6.00	-2.36, 7.90	0.14 (1.74)	0.9395
			Control	7	2.63 (2.53)	3.52	0.00, 5.35	0.29, 4.98		
	Week 4	Result	Intervention	4	2.29 (2.77)	1.78	0.00, 5.60	-2.12, 6.71	-0.65 (1.23)	0.6096
			Control	7	2.85 (2.68)	4.44	0.00, 5.42	0.37, 5.33		
		Change from Baseline	Intervention	4	-0.48 (0.72)	-0.20	-1.52, 0.00	-1.62, 0.66	-0.65 (1.23)	0.6096
			Control	7	0.21 (2.41)	0.00	-3.52, 4.76	-2.02, 2.44		
MMP-3	Baseline	Result	Intervention	4	6.50 (1.37)	6.96	4.54, 7.52	4.31, 8.68	-0.41 (0.63)	0.5374
			Control	7	6.90 (0.77)	6.82	5.40, 7.65	6.19, 7.62		
	Week 4	Result	Intervention	4	7.12 (0.84)	7.14	6.23, 7.99	5.79, 8.46	0.30 (0.82)	0.7247
			Control	7	7.02 (1.49)	6.84	4.91, 9.34	5.64, 8.40		
		Change from Baseline	Intervention	4	0.62 (1.02)	0.38	-0.32, 2.07	-1.00, 2.25	0.30 (0.82)	0.7247
			Control	7	0.12 (1.44)	0.17	-2.60, 1.87	-1.22, 1.45		
MMP-8	Baseline	Result	Intervention	4	11.32 (1.24)	11.40	9.97, 12.53	9.36, 13.29	-1.15 (0.65)	0.1117
			Control	7	12.47 (0.92)	12.54	10.92, 13.73	11.62, 13.32		
	Week 4	Result	Intervention	4	11.76 (1.05)	11.74	10.54, 13.03	10.09, 13.43	-0.36 (0.56)	0.5382
			Control	7	12.83 (0.93)	12.81	11.29, 14.05	11.97, 13.69		
		Change from Baseline	Intervention	4	0.44 (0.64)	0.70	-0.51, 0.85	-0.58, 1.46	-0.36 (0.56)	0.5382
			Control	7	0.36 (0.91)	0.30	-0.95, 1.73	-0.48, 1.20		
MMP-9	Baseline	Result	Intervention	4	12.52 (0.87)	12.49	11.63, 13.46	11.14, 13.90	-0.65 (0.36)	0.1068
			Control	7	13.17 (0.36)	13.10	12.73, 13.67	12.84, 13.51		
	Week 4	Result	Intervention	4	12.73 (0.76)	12.75	11.95, 13.48	11.52, 13.94	-0.01 (0.34)	0.9859
			Control	7	13.17 (0.48)	13.22	12.45, 13.68	12.73, 13.62		
		Change from Baseline	Intervention	4	0.21 (0.28)	0.29	-0.19, 0.45	-0.23, 0.65	-0.01 (0.34)	0.9859
			Control	7	-0.00 (0.56)	0.06	-1.09, 0.76	-0.52, 0.52		

Biomarker	Visit	Variable	Treatment	n	Mean (SD)[c]	Median	Min, Max	95% CI	Mean difference (SD)[b]	P-value[a]
MMP-13	Baseline	Result	Intervention	4	1.19 (2.39)	0.00	0.00, 4.78	-2.61, 4.99	0.02 (1.81)	0.9914
			Control	7	1.17 (3.11)	0.00	0.00, 8.22	-1.70, 4.05		
	Week 4	Result	Intervention	4	0.00 (0.00)	0.00	0.00, 0.00	N/a	0.00	N/a
			Control	7	0.00 (0.00)	0.00	0.00, 0.00	N/a		
		Change from Baseline	Intervention	4	-1.19 (2.39)	0.00	-4.78, 0.00	-4.99, 2.61	0.00	N/a
			Control	7	-1.17 (3.11)	0.00	-8.22, 0.00	-4.05, 1.70		

Table 57. Clinical Trial 2. Descriptive summary: Biomarker saliva samples MMPs, log scale, MITT subjects.
Biomarkers concentration is calculated as the average of repeated measurement. Log transformation is applied to the average concentration. Change from baseline is calculated from the log-transformed concentration.
ANOVA Model for baseline: Result=Treatment + error.
ANOVA Model for post-baseline: Result=Treatment + Baseline + Error.
[a]P-value F-test (Ho: Both treatments equal).
[b] Difference = Mean (SD) of the treatment difference (Intervention - Control).
[c] Mean biomarker concentration in pg/ml.

3.9.6 Global change scores

Symptoms were globally assessed at each follow-up visit to provide some overall context to the subjects' symptoms and also to provide validity to the OHIP data. Global change scores were recorded on a 5-point scale, responses were coded positively for improvements and negatively for deteriorations in symptoms where:

- Improved a lot (+2);
- Improved slightly (+1);
- Stayed the same 0;
- Become slightly worse (-1)
- Become a lot worse (-2).

Descriptive statistics were produced for each group at each follow up appointment listed in Table 58. At week 4 the mean global change with 95% CI was 1.03 (0.86, 1.38) for the intervention group indicating that the subjects in that groups felt that their symptoms improved slightly. The control group still showed a positive mean global change score but the confidence interval included zero at 0.26 (-0.02, 0.53) and therefore their symptoms may not have changed significantly from baseline. There were statistically significant differences between the two groups ($p<0.001$) at the 4-week follow up.

At week 20 the participants also reported improvement from week 4 in both groups, with greater improvement in the intervention group. The intervention group mean global score was 0.94 (0.56, 1.33) and the control group mean score 0.44 (0.07, 0.81). There were no statistical differences between groups at the 20-week follow-up ($p=0.067$).

Visit	Treatment	n	Mean (SD)	Median	Min, Max	95% CI	p-value
Week 4	Intervention	38	1.03 (1.03)	1	-1.00-2.00	0.86, 1.38	<0.001
	Control	40	0.26 (0.85)	0	-2.00-2.00	-0.02, 0.53	
Week 20	Intervention	36	0.94 (1.15)	1	-1.00-2.00	0.56, 1.33	0.067
	Control	40	0.44 (1.14)	0	-2.00-2.00	0.07, 0.81	

Table 58. Clinical Trial 2. Descriptive statistics produced for global change scores. Respondents were asked to consider their symptoms since the previous visit and indicate if they had improved a lot, improved slightly, stayed the same, become slightly worse or become a lot worse. Positive values indicate treatment improvement and negative values indicate deterioration in symptoms. Statistical analysis was carried out with MITT subjects using Mann Whitney U statistic, as the data were not normally distributed.

3.9.7 Effect sizes

The effect of treatment was examined using Cohen's *d* (Table 59). Interpretation of effect sizes differs but it is generally agreed that those values above 0.2 are seen to be having a small treatment effect, above 0.5 to have a moderate effect and above 0.8 to have a large effect.

Moderate treatment effects were seen in the intervention group for OHIP ordinal scores at week 4 (0.608) and week 20 (0.725). Moderate treatment effects were also observed in the intervention group for pain (VAS) both at the 4-week (0.517) and 20 weeks (0.700). Large effect sizes were observed in the intervention group for PI at week 4 (1.470) and week 20 (1.559). A moderate effect was seen in Escudier oro-mucosal disease index at week 4 (0.75) and a large effect observed at week 20 (1.01). Small changes were observed for the control group in OHIP scores and VAS scores at 4 and 20 weeks. No effect was observed in PI for the control group at any week. The effect sizes can be used alongside the surrogate measures of health (clinical indices) and subjective measures of health (OHIP) in an attempt to provide a comprehensive assessment of the effect of the intervention.

Outcome measure	Follow up	Group	Mean pre-treatment (SD)	Mean post-treatment	Effect size
OHIP-ordinal	Week 4	Intervention	49.66 (24.86)	34.55	0.61
		Control	49.39 (29.82)	42.25	0.24
	Week 20	Intervention	49.66 (24.86)	31.64	0.74
		Control	49.39 (29.82)	41.66	0.26
OHIP-dichotomous	Week 4	Intervention	6.55 (4.91)	3.03	0.72
		Control	6.71 (6.97)	5.30	0.20
	Week 20	Intervention	6.55 (4.91)	2.56	0.74
		Control	6.71 (6.97)	4.90	0.26
VAS	Week 4	Intervention	3.34 (2.07)	2.27	0.52
		Control	3.36 (2.23)	2.95	0.18
	Week 20	Intervention	3.34 (2.07)	1.85	0.70
		Control	3.36 (2.23)	2.49	0.38
Plaque Index	Week 4	Intervention	1.42 (0.36)	0.89	1.47
		Control	1.45 (0.34)	1.44	0.03
	Week 20	Intervention	1.42 (0.36)	0.86	1.56
		Control	1.45 (0.34)	1.47	-0.06
Escudier oro-mucosal disease index	Week 4	Intervention	31.12 (9.62)	23.93	0.75
		Control	27.97 (8.07)	28.46	-0.06
	Week 20	Intervention	31.12 (9.62)	21.40	1.01
		Control	27.97 (8.07)	25.65	0.29

Table 59. Clinical Trial 2. Unadjusted mean pre and post treatment values, standard deviations, and Cohen's *d* effect sizes.
It is generally accepted that *d* values of 0.2 represent small change, 0.5 represent moderate change and those >0.8 represent a large change. Negative values represent deterioration from baseline.

3.9.8 Correlation Analysis

Correlation analysis was performed to investigate the association of clinical outcome and oral health related quality of life. Spearman correlations between OHIP ordinal score over all domains and VAS pain score, overall Plaque Index, and oro-mucosal disease score (site score, severity score and activity score) were calculated.

There were significant correlations between OHIP ordinal scores and VAS pain score, the Spearman correlation coefficient being approximately 0.5 (except at Week 4), indicating moderate correlations between these two subjective measurements of oral health.

There were no statistically significant correlations between OHIP ordinal score and Plaque Index or between OHIP and the Escudier Index at baseline and Week 4. At week 20, the correlation of change from baseline between OHIP and Plaque Index is 0.30 (<0.01), indicating a small positive correlation. At week 20, the correlation of results and changes from baseline between OHIP and all three OMDS scores is in the range of 0.23-0.37 ($p<0.05$), indicating a small correlation between OHIP and OMDS.

3.9.8 Economic evaluation

Although oral hygiene aids were provided free as part of the trial this would not be the case in practice and hence an economic evaluation was undertaken to determine the cost-effectiveness of the intervention. Data were only collected from the intervention group subjects as the control subjects did not receive the intervention as part of the study protocol. The economic evaluation included the costs of the products provided and also the time taken to deliver the intervention excluding set up and surgery cleaning time.

The cost of the toothbrush was set at the current price (2012) of £95. The cost of the remaining interdental aids was estimated at £23.50 per subject. The costs of toothpaste provided to both intervention and control groups were ignored. For the cost benefit analysis it was assumed that patients would purchase toothpaste whether or not they participated in the intervention and that any additional toothpaste costs could be ignored.

The time input for the plaque control programme, delivered by a dental hygienist was estimated to be approximately 5 minutes. The total cost of an hour of patient contact time including all overheads for a dental hygienist working in General Dental Practice in the UK was estimated to be £51 (PSSRU, 2011). The cost for 5 minutes was then calculated at £4.25.

A cost-benefit analysis was carried out by comparing the costs including travel and time costs, with the benefits of treatment for the patients in the intervention group Patients in the treatment arm were asked to report estimates of travel costs including car parking. They were also asked to report the total time spent attending treatment sessions and their gross salary (in bands of £10,000). The assumption was made that patients worked full time for 1750 hours a year and hourly costs of patient time were estimated using the mid salary band value divided by 1750. Valuing the time of non-working patients is contentious but it is highly unlikely that these patients value their time at zero (Brouwer and Koopmanschap, 1998). Unemployed patients were assigned to the band £0 – £10,000 and retired patients to the band £10,000 - £20,000 to assign a value to their time.

The cost-benefit analysis assessed whether patients receiving the treatment judged it to be worth more than the cost. Patient travelling and time costs were added to the treatment cost (toothbrush + accessories + hygienist time) to determine the total cost of treatment for each patient. This value was subtracted from each patient's stated maximum willingness to pay (WTP) for the treatment to determine the net value of the treatment.

Private cost data and stated willingness to pay values for treatment were obtained from all intervention patients retained at the 20-week follow-up; all patients stated a positive maximum WTP value (mean £321, range £65 to £1500). Out of pocket costs for patients were generally small.

The net value of treatment ranged from -£97 to £1339. The mean value was £172 (CI £88 to £282); the median was £69 (CI £24 to £124); and the inter-quartile range was £2 to £194. Three quarters of the sample stated a maximum WTP in excess of the total cost of treatment, which was £122.75.

The cost-benefit analysis indicates that the benefit of the intervention perceived by patients exceeded the cost. The majority of patients in the treatment arm valued the treatment in excess of the cost, and the mean value was significantly more than the cost. Hence the cost-benefit analysis would indicate that the treatment is cost-effective when compared to no treatment. Without WTP data from the control group valuing their treatment the cost-benefit analysis cannot ascertain whether the treatment is cost-effective when compared against the control.

3.10 Discussion

This study aimed to provide a comprehensive assessment of the impact of plaque control in patients with the gingival manifestations of oral lichen planus. The total numbers of subjects previously involved in prospective studies exploring this relationship are few in number. The sample size in this study was considerably larger (n=82) and although the *a priori* estimate of 98 subjects was not achieved, sufficient numbers of subjects were enrolled to detect measurable changes within the study groups. A *post hoc* power calculation based upon the primary outcome measure OHIP, showed that the study was overpowered at 36 subjects per treatment group. One alternative method could have been used to determine sample size that of an adaptive trial design whereby interim analysis may have informed the numbers of subjects required at an earlier stage. This method is not without its opponents and was not carried out in this study as there is the potential for the study to incur bias by the knowledge of the interim results (FDA, 2010). Reasonable conclusions can therefore be made based upon the number of subjects recruited into the study.

This study also aimed to address some of the methodological problems that exist with many oral lichen planus intervention studies (Thongprasom *et al.*, 2011). This study builds on previous recommendations for assessing oral lichen planus interventions that usually account for patients' symptoms through VAS scores, and use clinical criteria scores.

The addition of a measure of oral health related quality of life, the Oral Health Impact Profile, a global transition score as well as those more commonly utilised assessment tools along with cost evaluations provide a comprehensive assessment of the intervention. This methodology could be applied to future oral lichen planus interventions and is not overly burdensome in terms of data collection or subjects' time.

Evaluation of the effectiveness of a personalised plaque control programme used tools that were both objective and subjective measures of health and disease and a combination of both (Escudier weighted index). The primary outcome measure was a change in OHIP score and discussion will initially focus upon the efficacy of the intervention and subsequently contextualise this with changes in OHIP scores.

The clinical evaluation of the intervention was based on Plaque Index (Silness and Löe, 1964) and the Escudier Index (Escudier *et al.*, 2007). In this study the mean Plaque Index scores reduced for the intervention group by 39.5% at the 20-week follow up compared to the control group, whose mean plaque scores increased marginally by 4.1%. Control subjects did not show any improvement at any week. The intervention was successful in reducing plaque compared to control at week 4 and was sustained to the end of the study at 20 weeks.

Early studies suggested that painful atrophic alveolar mucosa may discourage patients from brushing effectively, additionally it has been reported that powered tooth brushing can cause minor gingival abrasions (Erpenstein, 1985; Robinson *et al.*, 2005). The intervention therefore had the potential to exacerbate the lesions particularly with the friable, atrophic nature of the gingival tissue. It has been suggested that plaque removal would potentiate new lesions resulting from mechanical trauma, however this hypothesis lacked any evidence (Hermann, 1963). Contrary to the thoughts of Erpenstein, 1985, the results of this study showed that the personalised advice and products provided to the subjects facilitated improvements in plaque control; intervention subjects were not discouraged by any initial discomfort or bleeding. This acceptance may be in part due to the brush head design and method of action of the powered toothbrush that was used. This, coupled with appropriate interdental instruction may have facilitated less traumatic cleaning than either subjects' existing manual or powered brushing habits.

Previous studies have used various plaque indices and comparisons between studies are possible using percentage change from baseline and effect size. The personalised plaque control programme was comparable to improvements seen in two previous studies (Holmstrup *et al.*, 1990; Guiglia *et al.*, 2007). A further study by Lopez-Jornet and Camacho-Alonso reported greater reductions in plaque through a behavioural motivational oral hygiene intervention that was reinforced 1 month after commencement of the study (Lopez-Jornet and Camacho-Alonso 2010a). The duration of the Lopez-Jornet and Camacho-Alonso, 2010 study was only 8 weeks and it is uncertain if compliance would have been maintained beyond its relatively short follow-up.

In this study the reduction in plaque was maintained from week-4 to the completion of the study protocol at week-20. The treatment effect in plaque removal was large at both the 4-week (*d*=1.47) and 20 week (*d*=1.56) follow up visits. The effect size for PI reduction was greater than those observed by Guiglia et al (2007) using a combined regime of manual tooth brushing with a soft toothbrush using the modified Bass technique, interdental cleaning with dental floss and adjunctive topical corticosteriods (*d*=0.97) (Guiglia *et al.*, 2007).

The most common assessment of the extent of oral lichen planus lesions used a five point clinical criteria score subdivided into 6 areas of the mouth and lips (Thongprasom *et al.*, 1992; Thongprasom *et al.*, 2011). In this study a less widely used scoring system developed specifically for oral lichen planus was used (Escudier *et al.*, 2007). The Escudier index contains a subjective component (pain score) but the total score is weighted in favour of the clinically observed scores representing the involved sites and severity. Although using a different index has the potential to cause some problems with comparability of findings with previous oral lichen planus intervention studies, it does give a large weighting to the gingival involvement and was therefore the most appropriate to use in this study. The inclusion of effect sizes in the results attempts to maintain the comparability between studies. The results of the mucosal disease scores suggested that improvements were observed not only in in the number of sites affected, but the severity, and activity of the mucosal lesions in comparison to control ($p<0.001$ for all domains). Although the intervention was designed to reduce the inflammatory component in the gingival tissues, it was not intended to concurrently reduce the lesions affecting the remainder of the mouth. The reduction in supragingival plaque in the intervention group led to an improvement in the mucosal and gingival lesions as demonstrated by the significant reduction at weeks 4 and 20 in the individual and weighted Escudier oro-mucosal disease scores ($p<0.001$). The treatment effect was moderate at 4-weeks (*d*=0.747*)* and large (*d*=1.01) at 20 weeks for the intervention group. Whilst this study shows sustained improvement in the lesions to the end of the study, there was not complete resolution indicated by the final mean activity score (site x severity) of 9.50 (0.68) in the intervention group.

Plaque-induced gingival inflammation may play a synergistic role in oral lichen planus inducing local inflammation, which in turn is poorly controlled by the underlying immune mechanisms leading to epithelial desquamation. The precise mechanism by which this is occurring remains unclear. The findings give support to the conclusions of the original work by Erpenstein who proposed that the infection of the marginal gingiva gives rise to a non-specific inflammatory reaction that could sustain or even induce oral lichen planus lesions (Erpenstein, 1985).

Subjective assessment was carried out through OHIP and VAS pain scales to provide assessment of the impact that the gingival manifestations of oral lichen planus have on the patient. OHIP has not been used as a primary outcome measure for previous studies involving plaque control and oral lichen planus but is sensitive to change in a clinical trial setting (Allen *et al.*, 2001). A semi-quantitative system for recording symptoms was unable to detect small changes (Holmstrup *et al.*, 1990).

There were no significant differences at baseline for any domain ($p>0.05$) or ordinal OHIP sum score indicating similarities between the two groups at baseline. The mean OHIP ordinal sum scores at baseline were 49.7 for the intervention and 49.4 for the control group. In the original validation studies and development of the OHIP, subjects who were 60 years of age and over were evaluated. In this study the mean age of participants was 61.4 and therefore comparisons can be drawn between these original reference values and those in this study (Slade *et al.*, 1998). The mean OHIP for dentate subjects in the Slade study was 31.3 based upon a sample of 905. The subjects in this study reported more impacts at baseline indicating poorer quality of life.

Both intervention and control groups showed improvements in ordinal and dichotomous OHIP scores. When the treatment difference was accounted for (intervention - control), the overall effect was a statistically significant reduction in OHIP ordinal and dichotomous sum scores in favour of the intervention. The physical pain domain in OHIP contains questions that relate to the frequency that subjects experienced painful gums, sore spots and discomfort when eating. These symptoms are commonly reported in outpatient clinical settings therefore, it is reasonable to assume that this domain would have the potential for change. Although improvements in domain scores were observed, they were not

statistically different from the control group ($p>0.05$). The overall OHIP ordinal scores improved more than in the control group therefore the changes must lie in other domains. In the functional limitation domain there were significant differences between control and intervention groups ($p=0.0216$, $p=0.0137$). This domain contains questions relating to appearance, difficulty chewing, taste and digestion. It may be possible that improvements in clinical signs of inflammation then bring about these secondary outcomes measured in this and other domains. The largest differences between groups were observed with the psychological discomfort and physical disability domains. The psychological discomfort domain relates to being worried, self-conscious, miserable, concerned about appearance and tension. Perhaps the intervention is, by resolving the inflammation, reducing symptomatology with subjects consequently being less concerned about their oral health. There may also be some positive effect by which participating in the study affects this domain; particularly a study that monitors subjects more frequently than through their conventional clinical pathway. This may be particularly important when examining a cohort of patients with a potentially premalignant diagnosis (Holmstrup *et al.*, 1988; Mattsson *et al.*, 2002; Holmstrup, 2010). Within the physical disability OHIP domain which contains questions relating to being unable to brush teeth, avoidance of eating and unsatisfactory diet, there were statistical differences between the groups in favour of the intervention group at week 20 ($p=0.0035$). It is impossible to tell, without adjunctive further qualitative interviewing, which part of the intervention is the most important, the advice and reassurance or the provision of appropriate devices and aids that facilitate the perceived improvements in this domain.

Comparatively few impacts were observed in the final three domains: psychological disability, social disability and handicap. There were no statistical differences between the groups at either follow up visit ($p>0.05$) with the exception of the psychological disability domain at 20 weeks ($p=0.0324$). Perhaps this suggests that the gingival manifestations of oral lichen planus do not have large disabling effects but carry significant psychological impact associated with the diagnosis and chronic discomfort. Anxiety has previously been strongly associated with the initiation or oral lichen planus and frequent observation and monitoring during a clinical study may go some way to alleviating this anxiety (Allen *et al.*, 1986; Vallejo *et al.*, 2001).

The baseline pain scores in this study were similar to those of other lichen planus intervention studies. The baseline mean VAS was 3.4 whereas the range is wider in the literature lying between 2.2 and 7.7. It is generally accepted and despite there being slightly different thresholds, that VAS scores between 1-4 represent mild pain, 5-6 moderate pain and 7-10 represent severe pain (Serlin *et al.*, 1995; Farrar *et al.*, 2001; Jensen *et al.*, 2003). In this study the mean value represents mild pain however some subjects perceive their pain to be severe, with the maximum score of 8.1 recorded at baseline in the control group. The symptoms vary at an individual level and other subjects did not perceive any pain at all at baseline.

To ensure comparability of the findings, comparisons should be made to other oral lichen planus interventions which have been evaluated using change in VAS scale measurements as the primary outcome measure. Changes in VAS scores have not always corresponded with a change in the clinical extent of the lesions. Placebo controlled trials also report improvements in VAS scores for both intervention and placebo groups with few demonstrating a marked difference in post-treatment scores from baseline (Swift *et al.*, 2005; Chainani-Wu *et al.*, 2007). Those oral lichen planus studies reporting the greatest reductions in VAS score applied topical clobetasol propionate treatments and achieved VAS change scores in excess of 3.9 points for all formulations of the drug in comparison to the mean change of 1.6 observed in this study (Carbone *et al.*, 2009). Interpreting change also poses a challenge, with some advocating change above threshold values on the VAS scale whereas others use percentage change. It has been suggested that changes above 15% represent a noticeable change, above 33% a clinically meaningful change and above 66% a substantial change (Jensen *et al.*, 2003). In this study, a 1.6-point change in the intervention group at 20 weeks represented a 47% change in VAS score, however the control group also improved by 0.9 or 26%. There were no statistically significant differences between the groups at any week ($p>0.05$). The treatment effect (intervention-control) of 21% reduction supports the findings that there was a noticeable but not clinically or statistically significant difference between the groups. Despite these findings, pain remains an important symptom to measure and monitor over time.

In this study it was not ethical to discontinue subjects' concomitant medication prior to their enrolment in the study. Subjects' medication was not changed as part of the study and the baseline scores might have been influenced by previous treatment. Discontinuing treatment for any length of time prior to enrolment is likely to have exacerbated the lesions and symptoms and would have been challenged at ethical review. This would only have been possible if only previously undiagnosed patients were included in the study, and whilst strengthening the study findings in one respect it would have significantly lowered the numbers of subjects that would have been recruited potentially weakening its power. Unlike some previous studies the changes observed in the intervention group were not confounded by the concurrent initiation of topical corticosteroids at baseline. Whilst subjects were free to continue with their current treatment regimen (most frequently topical clobetasol propionate ointment 0.05% mixed with carmellose sodium 16.7% oral paste) the treatment effect was attributable to the oral health intervention.

The cost-benefit analysis indicates that the benefit of the intervention as perceived by patients exceeded the cost. The majority of patients in the treatment arm valued the treatment in excess of the cost and hence the treatment is cost-effective when compared to no treatment. Without data from the control group, the analysis cannot ascertain whether the treatment is cost-effective when compared against the control group.

In this study two sampling methods were applied to the collection of oral fluids; whole unstimulated saliva and GCF. The relatively small subgroup from which the GCF and saliva samples were taken reduces the generalizability of the findings of the biomarker analysis.

Within this cohort of predominantly middle-aged and older subjects there is the potential for local and systemic factors to confound the results and prevent meaningful conclusions from being drawn. Saliva has its benefits because it does not require any specialised equipment chair-side to measure volumes and concentrations are more straightforward to determine from a volume of saliva. There are, however, it also has significant problems in collecting saliva from subjects with hypo-salivation, who use concomitant medication, or suffer from other inflammatory conditions that may confound the concentrations observed

alongside the potential for anxiety and discomfort during 5-10 minutes of collection (Rhodus *et al.*, 2005). It could also be argued that collecting pure unstimulated saliva is not possible as the effect of collecting a sample may inadvertently be stimulating saliva production.

Tissue transudates have previously been collected directly from the oral lichen planus lesion and have been suggested to be the method of choice in erosive presentations. In this study serum transudate (GCF) as well as saliva was used to determine local levels of inflammatory biomarkers because of accessibility and proximity to the desquamative lesions. Screening for periodontitis was not carried out and does have the potential to confound the results of the GCF and salivary biomarker analysis. This is relevant as underlying periodontitis affects 45% of the adult population in the UK and at higher percentages in elderly populations such as those observed in this study (Steele and O'Sullivan, 2011).

The biomarker concentrations observed in the subgroup analysis exhibited no clinically significant difference between groups at baseline and follow-up. It is likely that the subgroup was not large enough to detect differences between groups. No conclusions should be drawn from the change following the intervention given the small sample size.

Previous studies have suggested that blocking pro-inflammatory cytokines or promoting immunosuppressive cytokine activity in oral lichen planus could be the target of future therapies. A large focus of recent research has been into the NF-κB dependent cytokines (TNF-α, IL-1α, IL-6, IL-8) in oral lichen planus, which were elevated when compared to healthy controls in saliva and tissue transudate (Pezelj-Ribaric *et al.*, 2004; Rhodus *et al.*, 2005; Rhodus *et al.*, 2007).

CD4(+) T-cell produced cytokines including IL-2 are required for the generation and maintenance of regulatory T-cells that provide protection from autoimmune disease. IL-2 was observed in relatively low concentrations in this study and in previous studies have not been detectable (Yamamoto and Osaki, 1995). They regulate growth, proliferation and differentiation of T-cells and themselves are produced by T-cells during an immune response. They promote the adaptive immune response against foreign antigens and pathogens (Sharma *et al.*, 2011). In autoimmune disease it is IL-2 and its receptors that are targeted in the treatment

of autoimmune disease. In particular corticosteroids, ciclosporins and tacrolimus, used in the management of oral lichen planus, aim to suppress the immune response. This may be achieved systemically or locally through inhibition of IL-2 production by activated T-cells, given that most subjects in this study were using concomitant topical corticosteroids. This may explain the low levels of IL-2 seen in the samples analysed.

Macrophage inflammatory proteins (MIP) are produced by a number of cells including lymphocytes, are known for their pro-inflammatory effects, and are chemotactic for leukocytes as such they could be a potentially interesting protein to observe in oral lichen planus. MIPs also synthesise other pro-inflammatory cytokines (IL-1, IL6, TNF-α), and elevated levels are likely to indicate higher levels of inflammation. There are also data implicating chemokines and in particular RANTES in the pathogenesis of oral lichen planus with T-cells themselves expressing RANTES (CCL5). This pro-inflammatory cytokine is chemotactic for T-cells, eosinophils, and basophils further attracting T-cells into the lesional area. It may also attract mast cells into the developing oral lichen planus lesion and stimulate mast cell degranulation. As this occurs mast cells release TNF-α which further stimulates and up-regulates RANTES secretion from T-cells. It has been proposed that this mechanism might be responsible for the chronic nature of oral lichen planus (Thornhill, 2001). As well as recruiting leucocytes into inflammatory sites, RANTES may also prolong the survival of inflammatory cells in oral lichen planus and further contribute to chronic disease. T-cell specific cytokines including MIP-1α and RANTES may be important in the recruitment of inflammatory T-cells into the connective tissue beneath the basement membrane, which is characteristic of oral lichen planus. There are also some non-specific immune mechanisms that are likely to contribute to the oral lichen planus lesions. MMPs function to degrade connective tissue matrix protiens and are regulated by the action of endogenous inhibitors. MMPs have been found in oral lichen planus lesional T-cells in much higher concentrations than would be found in healthy tissue supporting the findings in this study (Yamamoto and Osaki, 1995).

The differences found in biomarker concentrations in GCF and saliva and the higher concentrations of levels found in close proximity to the lesions give strength to using GCF as a fluid in which to analyse changes in inflammatory biomarkers in

patients with oral lichen planus. An alternative would be intralesional tissue transudate sample as previously reported (Rhodus *et al.*, 2007). Determining different levels of inflammatory mediators in GCF and saliva provides the opportunity to understand and monitor the therapeutic response to conservative or pharmacologically active treatments (Rhodus *et al.*, 2005; Rhodus *et al.*, 2007). This may ultimately lead to refinements in the available treatments that target specific parts of the immune response in an attempt to modulate that response rather than apply a more generic treatment aimed at symptomatic control. Further, comparisons could also be made with other chronic inflammatory diseases where therapeutics may target common inflammatory processes, but this requires consensus on reliable fluids to sample and standardisation of diagnostic processes.

3.11 Conclusions

A personalised plaque control intervention was effective in improving the oral health related quality of life and clinically observed gingival manifestations of oral lichen planus. The intervention was cost-effective and this study provides evidence to include intensive plaque control within patients' initial and on-going management. These findings are relevant to oral medicine specialists and periodontists but potentially have the greatest impact through general dental practitioners and dental hygienists. Successful translation of these findings requires a greater understanding of the barriers that exist to implementing research findings, these will be investigated further in Chapter 4.

Chapter 4

A qualitative study to investigate the perceived barriers to implementing clinical research findings in general dental practice in North East England

4.1 Introduction

Healthcare is facing an exponential growth in the volume of articles published each year and some estimates suggest that the knowledge base in medicine is doubling every 6-8 years. Researchers are encouraged by their institutions to publish in specialist journals, with high impact factors. For universities and other institutions they are an esteem indicator that relates to frequency by which articles are subsequently cited by other researchers. Impact factors do not, however, measure changes in clinical practice, arguably a truer impact, brought about by the results of the publication. Clinical impact is difficult to assess, unless the evidence is widely adopted or incorporated into national or international clinical guidelines. Selective publication in specialist journals risks disseminating the results of the work to a niche audience that, by its very nature, is not based in primary care. This strategy for dissemination risks isolating research from primary care where the vast majority of dentistry is delivered. An understanding of how dental practitioners engage with continuing education and how researchers should engage with practitioners is critical to bridging the divide between research and practice. If ignored and research cannot be easily translated into clinical practice, this divide is likely to widen.

The plaque control interventions that have been evaluated previously are potentially straightforward for dentists and hygienists to deliver. Personalised interventions have the potential to bring about improvements in oral healthcare and, even, quality of life. If these and other research findings are not accessible, understandable, or practitioners cannot relate to them, they are unlikely to be adopted into clinical practice.

Accessing journals can form a component of dentists' continuing education but a number of competing sources of continuing education now exist. The focus of this study was therefore to explore some of the difficulties that general dental

practitioners have in selecting reliable research evidence to support an evidence-based clinical practice. It also looked to determine which methods of continuing education and dissemination of research are the most effective. The results should inform clinical researchers, government organisations, regulators and manufacturers about how best to engage with dental practitioners, particularly in presenting and disseminating research findings.

4.2 Aim

The aim was to investigate the effectiveness of continuing professional development amongst general dental practitioners and to identify barriers to adopting new clinical evidence and applying this to their clinical practice.

4.3 Materials and methods

A number of methods have been described in the literature for qualitative research with healthcare professionals using questionnaires, meetings, workshops, focus group interviews, discussion groups and informal interviews (Baker *et al.*, 2010). This study used semi-structured interviews and a focus group with general dental practitioners to explore the aim of the study. The study was insured and risk assessed through Newcastle University with Zurich Municipal and reviewed externally by North of Tyne NHS Primary Care Trust Research and Development. The NHS ethical review process did not apply as patients were not involved in the design or conduct of the research.

4.3.1 Subjects

A purposive sampling method was used to ensure that the demographics of the interviewees and focus group participants were representative of general dental practitioners in the North East of England. This sampling method was preferred over convenience sampling to provide data applicable to the research topic that was relevant (Tong *et al.*, 2007). The demographics of the practitioners were stratified to include representation from: male and female general dental practitioners; within two years of their primary dental examination; those 2-10 years post qualification; and those qualified 10 years and over. This information was available through North of Tyne Primary Care Trust Performer's lists and the General Dental Council register. Potential participants were contacted at their practices and were invited to participate by letter and/or email. They were

provided with an approved participant information sheet and asked to consider if they would like to be involved in the study.

Fifteen potential participants were approached, thirteen accepted and two were unable to attend for the focus group date and time. In total, four, one-to-one semi-structured interviews and one focus group of seven participants took place.

4.3.2 Inclusion and exclusion criteria

The following criteria were applied to the potential participants before enrolment in the study:

Inclusion criteria:

- Hold a primary dental qualification (BDS/BChD or equivalent);
- Must be a current registrant of the General Dental Council;
- Currently undertake primary care dentistry in either NHS or private practice;
- Practise dentistry in the North East of England;
- Be willing to participate in one-to-one interviews or small focus groups.

Exclusion criteria:

- Does not practise or is retired from primary care dentistry;
- Not registered or currently suspended from the GDC register.

4.4 Conduct of the research

An initial topic guide was created prior to the first interview based upon a review of the literature. This was refined and added to following each one-to-one interview and prior to conducting the focus group. The constant comparative method (grounded theory) was used so that data collected were coded and analysed prior to conducting the next interview or focus group. This ensured that interviews were not conducted unnecessarily once a saturation point of ideas and concepts was reached (Glaser, 1965; Glaser and Stauss, 1967; Ritchie *et al.*, 2003).

4.5 Data management

Following review of the interview transcripts, major emergent themes (meta themes) were identified. Data transcripts were not returned to participants for correction but as part of the quality assurance process, participants were asked to comment on whether the conclusions drawn from the analysis were an accurate interpretation of the discussions that took place (member checking) (Lincoln and Guba, 1985). The resultant analysis was then entered into a framework to assist data handling and management with the aim to preserve the individual responses

and narratives that were described during the focus groups as well as report the emergent themes (Holmes *et al.*, 2008). Triangulation and confirmation of the emergent themes was carried out following independent review of the transcripts by a researcher experienced in qualitative methodology (peer examination), who was independent of the research team (Lincoln and Guba, 1985).

4.6 Results

Data saturation was reached following the four interviews and one focus group. Interviews were conducted in participants' workplaces with the focus group conducted at Newcastle University between September and November 2012. No other people were present during the interviews other than the participant and the interviewer. The interviews and the focus group discussions were recorded to digital audio (MP3) format and lasted between 27 and 53 minutes, with the focus group lasting 104 minutes in duration. These were then professionally transcribed verbatim. Participant identifiers were removed and replaced with codes detailed in Table 60. Four emergent meta themes were identified: peer review, postgraduate education, practice pressures and the relevance of research. Sub themes were also identified.

Participant ID	Gender	Position in the practice	Registration with GDC
ID1	Male	Associate	1990
ID2	Female	Associate and SPCDS Dental Officer (DO)*	2005
ID3	Male	Principal	2005
ID4	Male	Associate	2005
ID5	Female	Associate	2011
ID6	Male	Associate	2006
ID7	Female	Associate	2006
ID8	Female	Associate	2010
ID9	Male	Principal	2005
ID10	Male	Principal	1985
ID11	Male	Principal	1984

Table 60. Anonymised subject identifiers for general dental practitioners involved in semi-structured interviews and focus group.
*SPCDS Salaried Primary Dental Care Service.

Peer review

The strongest emergent theme was the importance general practitioners placed on their engagement and interactions with colleagues through peer review. These interactions took place in a variety of ways but most commonly occurred through informal discussion with other dentists during surgery hours and lunch-breaks. Its value was placed such that it was viewed as an integral part of everyday general practice.

"Before we make any changes the first thing I would do would be to seek peer review, so chat to other dentists in the area…peer review is probably the most important non-verifiable stuff." (Principal, ID3)

Obtaining a variety of opinions was important to be able to make an informed decision regarding particular treatment options, procedures or new materials. Some practitioners opinions were held in greater esteem than others with particular preference given to those with knowledge of general practice or themselves were experienced practitioners.

"I might go and speak to him and if he had certain feelings about it I might be swayed in one way than another." (Associate/DO, ID2)

Experience was highlighted as a particular strength in being able to distinguish the usefulness of new information. The adoption of new techniques and materials used by experienced practitioners provided further justification for less experienced dentists in their own decision making. Concerns were raised about the ability to implement certain techniques particularly if they were seen to be "academic". These comparisons also emphasised some divisions between different practising environments and the importance of selecting the most appropriate person to be involved with the peer review process:

"If one is in the hospital and one in practice they'll find it difficult to relate to each other…especially on cases and treatment plans, the barrier isn't the people it's the environment in which they work." (Principal, ID8)

In deciding how much weight to give to one view, comparisons were made between the person providing the opinion and the individual practitioners. Two distinct types of preference were expressed. Some practitioners favoured a 'true

peer', someone with similar scope of practice with whom they could easily relate to but at the same time were seen to be conscientious practitioners. Others were looking for an 'expert peer' with a different practising demographic to act as a local key opinion leader. These were people who were active in undergraduate and postgraduate education or experienced practitioners with private practice commitments.

A variety of other contexts were highlighted as opportunities for peer review alongside informal discussion with colleagues. These included: contacting former colleagues; participation in postgraduate courses; part-time teaching in dental schools; vocational training; practice study groups and involvement in national examinations.

Practitioners also felt that the information presented at postgraduate courses or conferences may be biased, with presenters favouring specific techniques or materials. Peer review was seen to triangulate the applicability of new information into practice.

"Sometimes people present all the positives of something but don't give you the pitfalls." (Associate/DO, ID2)

Particular formats of postgraduate course allowed greater opportunities for peer review:

"The ones with really long coffee and lunch breaks as you learn just as much talking to your colleagues in the break as the person standing at the screen." (Associate ID1)

There were perceived risks of not taking part in peer review, general practice was seen as somewhere that could be isolating particularly if the practitioner had been working in the same place for a number of years. The interrelationships between practitioners in different practices were particularly important to allow discussion and communication. There was a need to actively seek out interaction with other general practitioners and it was important not to become complacent about current practise. It was suggested that for those involved in vocational training, discussions with vocational practitioners formed a significant part of 'non-verifiable' CPD and was an incentive to maintain their knowledge.

It was acknowledged that the pressures of general practice could limit the opportunities for peer review. Other job roles that practitioners had previously held in secondary care settings were seen to actively facilitate interaction with other more experienced colleagues:

"When you are in a hospital it is very easy to pick up what is going on and current, you just have to be involved with the building." (Associate, ID6)

Postgraduate education

Continued professional development has been a legal requirement of General Dental Council registration since 2002 (GDC, 2012). It is delivered in a variety of formats, accredited qualifications, courses run by postgraduate deaneries and attendance at national and international conferences. Accreditation is provided by a number of publications for reading journal articles which may act to incentivise the reader. The success or failure of this format is essential to the current strategy employed by universities and researchers to disseminate the majority of research findings. Increasingly large numbers of providers are also offering courses either by formal taught programmes, single study days or through web based or online continued professional development. This meta-theme focuses on how successful these formats are and their associated problems.

Most postgraduate courses and conferences were not available free of charge, therefore the value (not necessarily the cost) of the course was important when deciding on whether to attend. This was most apparent with conferences, which would typically last between 2-3 days and be associated with higher fees. The benefit of courses or conferences conducted over a number of days was questioned:

"I'm sufficiently self-aware to recognise that after 3 o'clock on the first day I'm not taking any more in and so I'd have a day and a half where it was pointless me being there." (Associate, ID1)

"2 years ago…I probably learnt 3 or 4 things and it was £600…I decided that it wasn't a great use of money." (Associate/DO, ID2)

Two distinct types of conference were identified, those aimed at generalists such as the BDA annual conference and those aimed at academics or specialists such as

the British Society of Periodontology conference. The latter were not seen as being relevant to GDPs or a high priority. In general, there were mixed feelings about conferences, in particular with the breadth of topics covered. The more general form of conference was criticised for lacking focus and having a commercial bias towards marketing and cosmetic dentistry. It was felt that these were more suited to practice owners and improving the business of dentistry rather than improving clinical practice. Others felt that the focus and the level at which the presentations delivered in a conference were not always appropriate to their scope of practice.

"They were either pitched ridiculously easy so an hour and a half on fissure sealants or pitched so far leftfield, like aesthetic things that I'm sure are relevant to somebody but never anything we would do regularly…so either very straight forwards and no content or very leftfield and not applicable." (Associate/DO ID2)

"Maybe 50% of what's covered…I'm not saying that it's not relevant but it might be something that you might not want to focus on." (Associate, ID4)

The larger conference format was not the best delivery method for being able to learn with the choice of speaker and topic being particularly important to select. Smaller conferences e.g. Association of Dental Implantology whose focus was narrower were better received but it was acknowledged that the topic might not be universally relevant.

Practitioners felt that there was a limited amount of time in which to attend postgraduate education citing practice pressures and the importance of maintaining a work life balance with their families. Generally, it was felt that taking 2-3 days out of a working week to attend a conference was too much. Single or half-day courses were preferred as there was a limit to the amount of time spent attending courses outside of the 'core' subjects specified by the GDC. There was a reluctance to take more than one day off at time out of general practice and in particular that patients' appointments should not be cancelled. Despite being well delivered and received, the costs of attending some postgraduate courses were seen to be prohibitively expensive. Some felt that courses in London were inaccessible, with the initial cost of the course not being the problem but the additive costs of travel, accommodation and subsistence making them so. This was particularly the case for newly qualified practitioners and VDPs. Others would

travel further afield if they felt the value and relevance of the course justified the costs. Patients with particular conditions also motivated practitioners to attend postgraduate courses:

"I was thinking, you know, I need to know more about this so I can help my patients." (Associate, ID1)

Good organisation was required to be aware of which topics had been covered recently, an up-to-date personal development plan was seen as best practice. Five years was seen to be an adequate time period to cover most topics with the exception of medical emergency training. If carried out more frequently it was seen as more of an administrative exercise to fulfil the GDC's requirements.

"You wonder if you are going to take a day off work to recap what you have already been taught." (Associate, ID4)

The increase in volume of postgraduate courses and providers has led to difficulty in deciding which courses would be worthwhile attending.

"That has changed so much since I qualified, there has been an explosion." (Principal, ID11)

Reputation and experience of the speaker was an important consideration but the value for money of high profile speakers with national or international reputations was questioned.

Formal courses run by universities leading to qualifications e.g. MClinDent/MSc were seen to provide some structure to continuing professional development as well as providing long term career benefits. Despite being expensive, some viewed this type of course as a passport into a more mixed or private practice. The variety of delivery methods used by these programmes, including ample opportunities for peer review, would appeal to a variety of learning styles. There was also a feeling that many of these courses were directed towards younger practitioners and formed part of a newly emergent career structure in general practice.

"BDS isn't enough anymore, you have to continue doing exams to stay one step ahead of the competition." (Associate, ID5)

The format by which postgraduate courses were delivered was important at an individual level with mixed preferences discussed. Discrepancies were highlighted in the perceived benefit provided by hands on courses. The practical elements of some dental courses were criticised for not being delivered in the most appropriate way and did not always add value to the course.

"You were asked to prepare a cavity and fill it with this new material when in effect all I was doing was what I do a hundred times a day…it didn't improve my ability to do the task." (Associate, ID3)

It was important that if a hands-on element was provided then it should be the primary focus of the course otherwise it could be seen as an afterthought. The ability to see the presenter carry out a technique by a live demonstration allowed practitioners to make direct comparisons and appraise their own techniques. If this was not possible then recorded and edited video was seen as a good alternative. It was important for the speaker to have had experience of general practice to demonstrate that the content was relevant. Any practical or small group teaching should be informal enough to allow the presenter to interact with the participants and provide feedback on their work. Essential topics such as cardiopulmonary resuscitation updates were best delivered through "real life" scenarios using simulators or with their team in their own practice.

Day symposia also received a mixed response. They were seen by some as less effective than small interactive groups but more beneficial by others because of the opportunities for peer review. They also gave practitioners a sense that they belonged to part of a wider dental community facing similar problems. Courses run during the normal working week were associated with a double cost, one for the loss of a day's self-employed earnings and the other with the course fee.

Most practitioners regularly accessed online content associated with verifiable CPD from the journals that they subscribed to, most frequently Dental Update and the British Dental Journal (BDJ). Recognising that that there would be questions associated with particular articles was an incentive to read them. A number of other online sources were discussed with some requiring annual subscriptions (Dental Juice, Dental Channel, CopDend, Tipton Training). Younger practitioners were seen to be more accepting of, and engaged in, a higher volume of online CPD

whereas older practitioners preferred interaction with colleagues and the opportunity for peer review. The volume of verifiable online content was seen as being a positive but the resources were not always presented in the most appropriate formats. It was seen as being time and cost-effective but *"soul destroying" (Associate, ID1)* as the delivery did not promote a change in behaviour or practice. One significant disadvantage was that there was no summary or notes from which to refer to or reflect upon after the event.

One successful format of online CPD was through webinars that allowed practitioners to connect via the internet to a speaker. This environment was seen to be 'safe' in that they felt comfortable asking questions in this format either verbally through a microphone or typed onto a shared screen. It was a potentially cost-effective way of seeing speakers without the expense of taking time out of practice. Online CPD also allowed practitioners who were taking a career break to access up-to-date content and maintain their GDC registration.

Practice pressures

General dental practice was viewed as a busy, high-pressured environment in which to work, almost all participants found that one of the greatest pressures in general practice was time.

"Dealing with the patients and treatment itself is quite taxing on the mind and during lunch time and after hours you're then dealing with staff and the management of the business and all the problems with that." (Principal, ID3)

The extent of these pressures meant that some practitioners felt that taking time out to attend postgraduate courses during the working week was either problematic or not feasible.

"I don't find time to take a day off to go and do a course for myself…I can't see that changing." (Principal, ID11)

Others felt that these pressures were increasing in number particularly in NHS practice because of contractual obligations and NHS targets. Although taking time out of practice would be preferable, they felt that their targets were equally if not more important to reach.

"It's getting more arduous to get it all in." (Associate, ID1)

"I don't find the time to take a day off and do a course for myself…I can't see that changing." (Principal, ID11)

It was for this reason that one participant felt that committing to a formal taught postgraduate course forced them to take time to commit to their own development:

"I felt I needed to commit to something to make sure I kept up to date with my CPD, I felt if it was off my own back, it was difficult, because you are so busy in the practice working 9-5 head-down its difficult unless you make time and commit to something to keep up-to-date..." (Principal, ID3)

Potential barriers existed within individual practices to implementing novel techniques. These directly related to the employment status of the practitioner with distinct differences between associates and practice owners. This study highlighted that the owners or dental corporates are the gatekeepers of techniques and materials that are used in primary care and themselves are potential barriers to changing behaviour of their colleagues. Principals generally wanted to trial and evaluate materials as well as assess the impact of the costs prior to deciding to order them for the practice.

If the practice owner deemed the cost to be prohibitively expensive or the material was considered not essential then the associate would have to personally purchase additional materials. Whilst some were prepared to do this, others were not limiting the availability and use of the most-up-to-date materials and techniques. Principals also felt that this was a potential source of conflict within the practice:

"I'd certainly feel more comfortable using new things myself before saying the associates have to start using it…Depending on outlays I'd be quite keen to try it myself before buying it for everyone. I wouldn't like associates to all be having different materials, as you want everyone using the same. Some associates do like to buy things themselves but it causes problems with other associates saying they want to use it as well but don't want to pay for it." (Principal, ID9)

The most up-to-date materials were seen by associates as being beyond the reach of NHS primary care dentistry. Private practice was seen to be more flexible in

ordering of materials as any increase in overhead could be more easily passed onto the patients.

Some also felt that as a result of the economic downturn, patients were seeking and demanding of cheaper alternative treatment options. For example, some were not willing to pay for crowns and practitioners were being forced to place larger plastic restorations and required suitable alternative materials.

"I found that they were no longer suitable for amalgam fillings, patients were less willing to pay for expensive crowns and prefer to try fillings, so, I found myself doing an awful lot of posterior composites." (Associate, ID3)

One way of becoming aware of new materials and techniques was through practice visits by manufacturer's representatives. The dental industry and to some extent clinical researchers are reliant upon manufacturers' representatives engaging with primary care practitioners in the dissemination of products and research findings. They were seen as being useful to make practitioners aware of new products, obtain product samples and provide verifiable CPD for nurses. There were some strong feelings against them largely because of the inability to relate the handling properties of materials and the clinical applicability. Another criticism was the perception that the representatives had only sufficient knowledge to sell the product.

"If you wanted to be a smart alec and wanted to give them an uncomfortable time you could tie them in knots very quickly." (Associate, ID1)

The small community of dentists meant that practitioners were aware of who the more knowledgeable representatives were, despite this they remained sceptical of their opinions because they were paid on commission. Dentists would be seen to be more reliable representatives.

Contractually, practitioners working under the regulation of an NHS contract had an obligation to carry out clinical audit. The areas that were evaluated were usually ones that practitioner's had specific interest in or wanted to evaluate the success or failure of a new technique or material. Most felt that it would not have been possible to conduct an audit during surgery hours because of time constraints and pressures from principals to maintain productivity and achieve NHS

contractual targets. There was a perception that both the Salaried Primary Care Dental Service and hospital posts allowed more flexibility to be able to carry out audit within their practicing day. In general practice there were no incentives to carry out audit, particularly working as an associate and more commonly informal *ad hoc* self-appraisal took place. Practitioners would change their behaviour more readily based on information they had received on a course or through peer review rather than formally auditing their current practice.

The Health and Social Care Act 2008 changed the way in which primary care dentistry was regulated; from April 2011 all primary care dental practices were regulated by the Care Quality Commission (CQC). The CQC being an independent regulator of all health and social care in England, its role is to measure the quality and safety of the care provided against a series of essential standards. Practitioners were concerned about the power and the focus of their new regulator:

"There is a fear factor within primary care dentistry of the CQC…the CQC is having an effect on the focus of the practice as its aimed so much towards satisfying the CQC requirements, which in terms of patient care and quality are way down the list of what they're looking for – they've got rid of Dental Reference Officers…There is somebody that will come in to check you have got a vulnerable adults policy which the cleaner knows about and have signed at the bottom that they know about this policy but there isn't anybody checking whether you're leaving apical radiolucencies and discharging sinuses everywhere – strange times." (Associate, ID1)

Practitioners also followed a number of national guidelines such as those published by the National Institute for Health and Clinical Excellence (NICE), Cochrane collaboration, NHS delivering better oral health: An evidence based toolkit for prevention and from the BDA. Some of these guidelines were criticised for either being too difficult to understand (e.g. Cochrane) or were sometimes based on opinion rather than evidence (e.g. NICE). As a result practitioners would judge if the guideline had a high strength of evidence, if lacking, they would rely upon their own experience and only use them if they were deemed relevant.

Relevance of research

Practitioners had difficulty relating the application of scientific research into general practice, perhaps in part due to the difficulty in understanding and interpreting research papers:

"There wasn't much exposure to research papers while I was at University…it was only when I began to do my own postgraduate training I learnt how to interpret them." (Associate, ID3)

The majority of practitioners accessed printed dental journals, those most commonly mentioned were the British Dental Journal (BDJ) and Dental Update along with magazine style publications such as Dentistry and the Dental Tribune. These were a good source of CPD in conjunction with the online components of the Journal.

The BDJ was seen as a credible publication because the articles had been peer reviewed, some saw it as the authoritative text for the UK profession. Its broad range of topics was generally seen as a positive aspect of the publication, but despite this it was seen as being too academic for some practitioners. The main criticism of the articles published in the BDJ was that the settings that research was carried out in were usually university teaching hospitals and therefore not always relevant to general dental practice.

"Maybe they've been carried out in universities in a very controlled way and that might not be necessarily applicable to your practice." (Principal, ID 9)

It was thought that the rigorous process for submission and acceptance of articles might not allow the journal to become practitioner friendly. It was acknowledged that the format had changed over the last 10 years in an attempt to become more relevant.

"The BDJ is a lot better than it used to be, it used to be criticised for being very academic but is developmental now and in 10-years' time I think it could be very GDP friendly." (Principal, ID11)

Suggestions were made to improve the relevance further:

"At the end of it [article] they have a list of questions, why did you do this research and possibly there could be something from a GDP saying how it is relevant to general practice." (Principal, ID11)

The format and content of Dental Update was preferred to that of the BDJ, in particular the straightforward, clear manner in which the information was presented further, it was perceived to be more practice focussed and therefore relevant. Illustrated clinical application of techniques and materials was also preferred to graphs and diagrams which could be difficult to understand or interpret.

Access to other journals was difficult unless the practitioner had an affiliation to a teaching hospital or university. Other publications including the *Journal of Paediatric Dentistry*, *Disability, and Journal of Clinical Periodontology,* were criticised for having a limited amount of relevant material in them to general practice. It was thought that these were more relevant to academic or SPCDS roles. One practitioner also felt that they were not intelligent enough to understand the relevance of specialist publications:

"Even then you might only find one article that is semi-relevant to you. I'm sure if I was cleverer it would be relevant." (Associate/DO, ID2)

Practitioners engaged with the printed press in different ways, some would focus solely upon the articles that were associated with verifiable CPD whereas others would read all of the articles in an issue of that journal. Most commonly practitioners would make a judgement about the relevance of an article based upon the title and abstract. Abstracts were seen as usually being sufficient upon which to base an opinion, if the abstract indicated that the article might change current practice then the full article would be read.

"You can either skim to get the answers or if you find that it actually looks interesting you can read the full article." (Principal, ID11)

"So just to keep up with the CPD I would read the articles, but also as a proportion of the journal that I read, which is relevant to myself, it's probably quite a small proportion, quite small, maybe 20%." (Associate, ID4)

"I'll read the title of the abstract and them jump to the conclusion to see if it can be applied to my work." (Associate, ID7)

"I tend to read it cover to cover but if there is anything particularly dreary I tend to miss that out but generally I do read most of it." (Associate, ID2)

Accessibility to journals was important, despite having a printed copy of the journal delivered to either their home or practice this didn't always mean that they were read. Access to the online version of the journal meant that practitioners could be more selective about the articles that they read.

"I suppose the thing is they come through your door so you are probably going to look at them, but you can also find you've got however many BDJs still with the cover on that you haven't opened at all. So the thing is nowadays with computers and information [is available] instantaneously online." (Associate, ID1)

Practitioners perceived the evidence base for much of what is done in general practice to be inadequate and that it could be improved. Involving practices in research was seen as a positive step but it was seen as a difficult place in which to conduct research in because of the time pressures involved. Despite this some practices were involved in or had been invited to participate in practice-based multicentre research studies such as FiCTION, IQUAD and INTERVAL. There were financial incentives to the practice owners for being involved in the research studies

"We joined the (NAMED) trial for financial reasons. The topic was quite interesting but it was the financial part as a practice owner that will make you more keen to do it." (Principal, ID9)

Associates were not involved in deciding if the practice would be taking part in research studies and therefore not aware that they were taking place. The incentives that practice owners received were not passed on to the associates.

"We've just started dong the (NAMED) trial as well and as an associate we don't get paid any of the money awarded to the practice for doing the research. We were asked if we would take the time out of our day lists to do it but I opted out as I would get behind with my target for no financial gain even though I was quite interested in the topic of research." (Associate, ID5)

Practitioners felt that in the short term that research articles that were published should be written specifically stating the relevance to general practitioners and in the longer term further involve practices in new studies. Research institutions could also be more dynamic in the way that they engage with dental practices, this could include providing summaries of recent research studies to practices, or hosting annual update events for practitioners to attend.

4.7 Discussion

In 2002 the General Dental Council (GDC) introduced a legal requirement for all dentists to carry out a minimum of 250 hours of continuing professional development in a 5-year period. 75 hours of which must be verifiable, this in practical terms requires a certificate of completion of the activity which itself must have pre-published aims and objectives, anticipated outcomes and quality control measures (GDC, 2012). Further changes occurred in 2008 when the GDC registered dental nurses, and technicians introducing CPD requirements for these groups for the first time. This legislation imposed by the professional regulator recommended core CPD topics, however these may differ from an individual practitioner's learning needs (Bullock *et al.*, 2010). These requirements have led to an industry wide exponential increase in the volume of CPD providers with significant challenges in selecting material that is relevant, beneficial and practitioners are able to identify innovations that have meaningful impact to their clinical practice.

There is relatively little knowledge of the effectiveness of CPD interventions, this may partly explain the importance that practitioners placed on peer review in this study (Sohn *et al.*, 2004; Rivas *et al.*, 2012). Triangulation of new information with their own and others current practice appears to be important in examining the applicability and validity of new information (Schostak *et al.*, 2010).

Despite the previous Government recommendations and inclusion in previous NHS dental contracts, there is little evidence in dentistry that peer review is useful. Publications prior to the general dental services (GDS) contract change in England and Wales in 2006 had more modest news about the importance of peer review (Health, 2001a; Watt *et al.*, 2004). Dentists were also criticised for appearing to lack the critical and evaluative skills that are required for audit and peer review (Watt *et al.*, 2004). The pre-2006 arrangements encouraged dentist participation and the contract reimbursed practitioners for undertaking audit and structured peer review (Health, 2001a). The 2006 GDS NHS contract removed the contractual obligations and financial incentives of audit and peer review, the role of clinical governance then fell under the requirements of the Primary Care Trusts (Health, 2005b). Evidence suggests that financial incentives will incentivise participation and bring about changes in practice behaviour although it may not necessarily

improve patient care (Flodgren *et al.*, 2011a). With the removal of this formal peer review process, practitioners now rely upon their own networks to engage in peer review. This presents a challenge for researchers to promote an evidence-based approach, particularly if practitioners feel that they could not relate to a hospital or university-based practitioner. Evidence published suggests that periods of structured peer review in medicine are positive and can lead to a service improvement. Hospital and university practitioners could then bring a different skill set and perspective, further enriching the process. The reliance upon small networks to undertake their own peer review risks the perpetuation of a limited knowledge pool with access to limited resources. It also risks being influenced by stronger characters within a group who may have particular opinions supported only by personal experience rather than evidence. The importance placed upon it in this study could strengthen the support for regional or national organised audit and structured peer review in the future (Cannell, 2012). There is the potential for the finding of clinical trials of relevance to general practitioners to be discussed and evaluated as part of a formal peer review structure. This may deepen the understanding of how clinical trials are conducted in hospital and university settings and begin to forge some local networks by which the final step of evaluation could be conducted in general practice.

The concept of a career structure in General Dental Practice appears to be emerging and becoming more important to younger practitioners. A formal career pathway through the Faculty of General Dental Practitioners (UK) is one example but the plethora of privately run courses mean that less formal routes of career development exist (FGDP(UK), 2008). In 2001 the Department of Health recommended that all healthcare professionals had a personal development plan (PDP) to structure their career development (Health, 2001b). Planning of continuing professional development in this way has been shown to enhance its benefit (Bullock *et al.*, 2007; Eaton *et al.*, 2011). This study suggests that personal development planning is encouraged immediately post-graduation and forms a part of foundation training but it is not widely used after this period. PDP planning may become an increasingly important role for postgraduate tutors in the future to ensure that the potential of these tools are fulfilled, and that the CPD carried out by practitioners remains relevant and patient and practice-centred (McGlone *et al.*, 2001; Evans *et al.*, 2002; Wright and Franklin, 2007). A career structure may also

become more relevant to general practitioners as the GDC introduce their revalidation process for practitioners' registration (Jennings, 2007; GDC, 2011).

Postgraduate courses leading to qualifications were seen to be important to younger practitioners supporting the findings of other studies investigating trends in dental CPD (Leggate and Russell, 2002). The impact of a sustained period of postgraduate education has been viewed positively in this and previous studies. Practitioners viewed this as having the potential to alter the type of treatment they offer and change their scope of practice (Calnan *et al.*, 2000; Silvester *et al.*, 2000; Bullock *et al.*, 2009). This may be a reflection of an increasingly competitive employment market in primary care, possibly as a direct result of the numbers of graduating dentists in the UK (Smith, 2011). Competition may increase further with the opening of a new privately funded dental school in the UK and the introduction of direct access to dental hygienists and therapists by the GDC (Smith, 2011; Buckingham, 2012, GDC, 2013).

Practitioners found the most successful type of postgraduate course to be one that they believed had direct relevance to general practice and was interactive, these findings were supported by a previous systematic review into continuing medical education (Cantillon and Jones, 1999). Previous studies have suggested that local courses run at a deanery level were provided at no cost to participants, although courses are subsidised, practitioners make partial payment towards costs of the course (Bullock *et al.*, 2010). Practitioners were self-selecting about the courses they attended and also factored in costs, and it has been suggested that this method reinforces current knowledge and risks failing to address deficiencies (Firmstone *et al.*, 2004). The format by which courses were delivered was important at an individual level with previous studies supporting hands on elements (Christensen, 2004; Watt *et al.*, 2004). This study highlighted mixed views about the delivery and quality of these formats unless they were the primary focus and the structure allowed close observation and appropriate opportunities for feedback.

Similar strengths and weaknesses of printed materials (journals) were outlined to those found previously (Bero *et al.*, 1998; Watt *et al.*, 2004). Those most commonly read included the British Dental Journal and Dental Update. The limited numbers of journals that were subscribed to suggested that generally practitioners

did not have access to specialist level publications. This may be by choice in that they did not feel that the publications were relevant or because they did not see any potential benefits from additional subscriptions. The limited access to a variety of dental journals potentially results in large numbers of clinical trial findings being inaccessible to general practitioners. This raises the issue of whether the research community should support open access publications and in doing so allow more practitioners to read the work. The costs of allowing such access are likely to be prohibitively high at US$3000 per article. Practitioners criticised the volume of printed literature and the applicability of the content of some journals to everyday clinical practice with particular reference to the settings within which the research was conducted (Bero *et al.*, 1998). Open access could lead to further criticism of journals with an even greater volume of material being available. Journals had little direct impact on changing behaviour and there is little evidence that printed or online journals generally do so (Bero *et al.*, 1998). The British Dental Journal in particular being viewed as less practice friendly and more academic (Watt *et al.*, 2004). The expansion of online CPD allowed participants to more easily obtain verifiable credit for the journals they read. Researchers conducting clinical trials therefore must be mindful of these barriers and be creative in disseminating findings using a variety of media including journal publication. The results suggest that specialist publication alone will however not translate findings into practice. Conferences were also seen as poor value-for-money in relation to the learning experience, supporting the conclusions of a systematic review into continuing medical education that suggested that didactic teaching and printed material (journals and guidelines) had very little impact on changing practice (Bloom, 2005).

The difficulties for dentists undertaking a career break have previously been described (Leggate and Russell, 2002). The advent of online CPD appears to have made maintaining registration easier to achieve and the increasing quantity was a positive feature. This is likely to become increasingly important as the demographic of the profession changes in the future (McKay and Quinonez, 2012). One significant shortcoming of a large proportion of online CPD was way in which it was presented, it was felt that the format did not lend itself to changing clinical practice and there was no reinforcement of the information after the event. Positive online formats were webinars that allowed interaction with a remote

speaker in a non-threatening environment. It is perhaps this format which could be engaged with further by clinical researchers.

Patient-based factors were a motivational incentive for practitioners to seek out more information about certain topics, something that has be shown to have variable effectiveness in previous reports (Bero *et al.*, 1998). The potential impact of the worldwide economic recession may also impact on the choices that patients make in terms of their healthcare, with treatments delayed or practitioners being forced to make compromises about their treatment away from perceived best practice (Vujicic *et al.*, 2012).

Previous studies have suggested that local primary care research networks are examples of best practice in primary dental care research, noting the successes of the Prep-panel and Birmingham Research in Dental General Practice. Both exemplify how research can be accomplished in primary dentistry (Hopper *et al.*, 2008). The potential for some practitioners within the practice to be unwilling to participate in research has not been previously reported, with some practitioners fearful that their involvement would result in failure to fulfil their contractual obligations, particularly within NHS practice.

The outcome measures for continuing professional education interventions vary widely, this has led to problems in systematically reviewing these outcomes and leading to problems with meta-analysis (Bero *et al.*, 1998; York, 1999). Academic institutions will in the future be competing for smaller sums of funding and will be assessed by the true impact of their research as well as its scientific originality (REF, 2012). Researchers should be mindful of this in the design, conduct and disseminate their research findings in a manner that is likely to elicit a behaviour change for healthcare improvement. Future research should investigate the true cost-benefit obtained from the different formats of CPD as well as focussing on the underlying theory of behaviour change (Bero *et al.*, 1998; Eccles *et al.*, 2005). This may mean a change away from the printed press to more intensive and dynamic formats of continuing education (Freemantle *et al.*, 1997; Bero *et al.*, 1998; McGlone *et al.*, 2001). No evidence was found evaluating the impact that the change in healthcare regulator has had on the educational needs of General Dental Practitioners.

4.8 Conclusion

This study highlighted the challenges that general dental practitioners face in selecting and engaging with postgraduate education. Informal peer review was seen as being particularly important in the triangulation and evaluation of new information and techniques. Generally practitioners accessed a limited number of dental journals that were not of a specialist nature. Barriers remain to the successful translation of contemporary clinical research unless dissemination occurs outside of these specialist publications. Collaborative research partnerships incorporating primary dental care, may begin to challenge some these barriers between researchers and practitioners and foster a translational approach.

Chapter 5

Discussion

Translational research involves the transfer of knowledge from the laboratory into a clinical situation where an intervention and can be evaluated and accepted. In this way, research findings can be successfully implemented to bring about improvements in health for the wider population. The investigations reported in this thesis typify translational research, and demonstrate impact in understanding the underlying processes of health and disease (Figure 40).

Initially, established gingivitis was used as a model to evaluate and develop a personalised plaque control programme. This model brought together traditional clinical monitoring techniques and pioneering laboratory technologies to evaluate sequential plaque control interventions. Having established the efficacy of the intervention, it was adapted, and subsequently applied to a new clinical situation: the gingival manifestations of oral lichen planus. Comprehensive evaluation of the intervention was necessary incorporating measures of clinical effectiveness alongside those that would capture changes in symptomatology. In patients with painful and refractory disease, this necessitated the use of outcome measures which could capture changes in pain and quality of life. Clinical observations recorded changes in plaque and extent of the disease providing information as to the clinical effectiveness of the intervention. Clinicians and those commissioning healthcare services must ensure that limited financial resources are used to the greatest effect. Health economic analysis was undertaken to determine whether patients placed value on the intervention in excess of the costs of its delivery. The final step in the transfer of knowledge is from research finding into practice. In dentistry the general dental practitioners are the key stakeholders in delivering healthcare. Understanding the barriers that exist in disseminating, accepting and implementing recommendations is of utmost importance to facilitate changes in healthcare delivery. An investigation was undertaken to evaluate the attitudes of general dental practitioners towards research and continuing education in an attempt to identify the most successful methods for bringing about these changes.

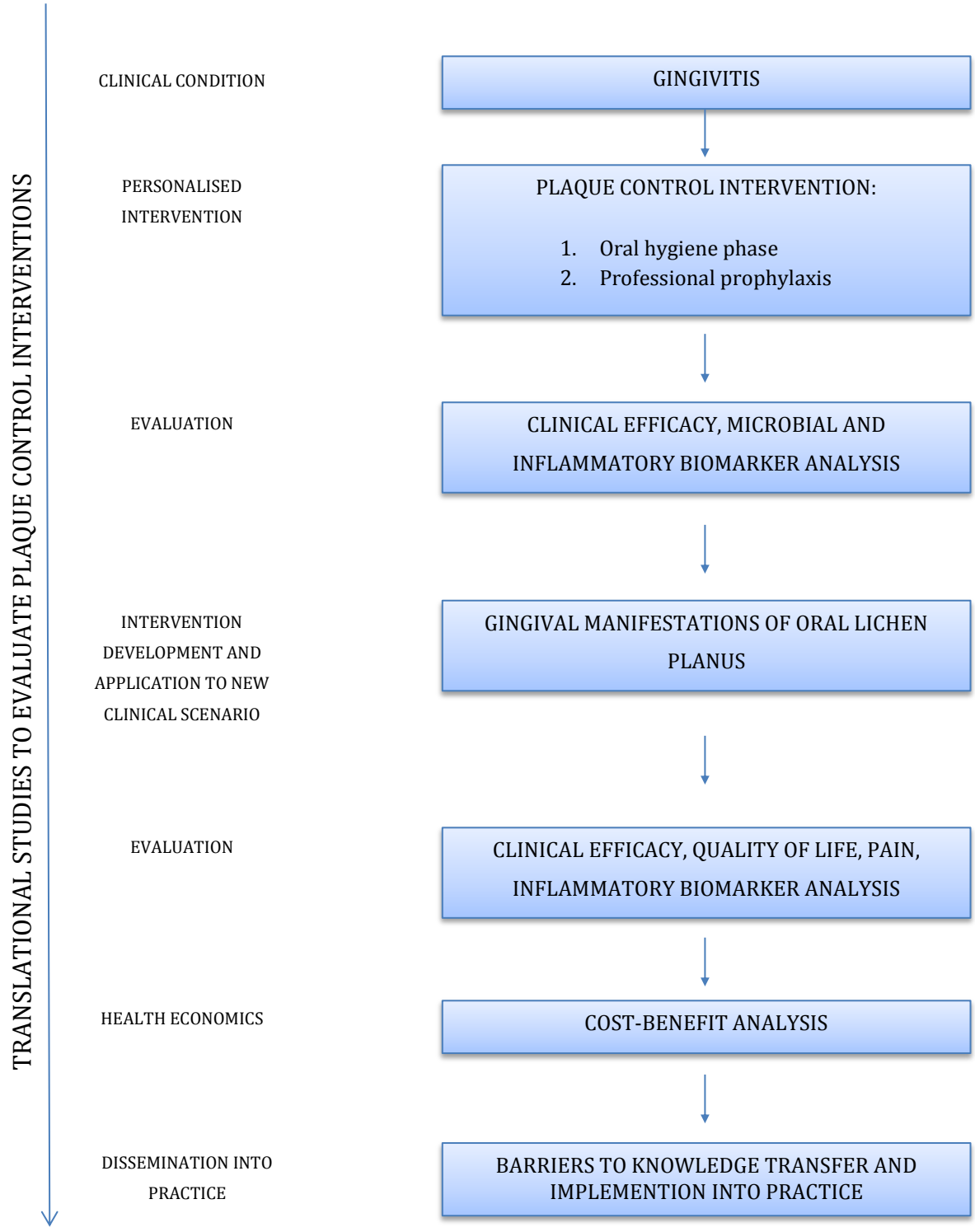


Figure 40. Overview of the translational studies reported in this thesis.

In the established gingivitis model, the intervention of a powered toothbrush and subsequently a professional prophylaxis brought about the expected resolution of the clinical signs of gingivitis. The laboratory analysis using multiplexing technologies enabled the detection and quantification of multiple biomarkers and microbial pathogens from relatively small sample volumes of GCF and plaque.

The pathogenesis of established gingivitis in this model allowed the use of host-derived biomarkers to be evaluated in conjunction with bacterial profiling. The presence or absence of specific inflammatory mediators or bacterial species may themselves be indicative of health or disease. Interactions occur between pathogenic microorganisms functioning in complex biofilms and host derived biomarkers that function as part of networks of inflammatory stimulation and suppression (Preshaw and Taylor, 2011). The pro-inflammatory cytokines indicative of inflammation have been the focus of significant research, particularly into the pathogenesis of periodontitis. A number of other host-derived proteins, inflammatory mediators and tissue breakdown products have previously been analysed in both gingivitis and periodontitis (Buduneli and Kinane, 2011). In the gingivitis model, it has been possible to detect a number of host-derived mediators of inflammation using multiplexing technologies. Not all of these are likely to be predictors of progressive disease and some may only be expressed transiently in different phases of inflammation.

In this established gingivitis model, the clinical measures showed improvement following sequential plaque control interventions with the biofilm profile changing towards one of more commensal and aerobic species. Only small, transient changes were observed in the host-derived mediators of inflammation. These findings challenge other outcomes that might be expected in response to the non-surgical management of chronic periodontitis (Kinane *et al.*, 2003; Buduneli and Kinane, 2011). These transient changes may represent tissue remodelling at levels below the threshold for tissue degradation. Ultimately it is the response of the tissues to microbial endotoxins that initiates gingivitis whilst local genetic expression and modifying systemic factors are most likely to be responsible for the severity and magnitude of the response.

The profiling of samples from patients with different stages of gingivitis (and subsequently periodontitis) may ultimately lead to the establishment of some

threshold values above or below which, destructive disease may be predictable (Kinane *et al.*, 2011). Furthermore, correlating and quantifying the levels of bacterial species present, with host-derived biomarkers, may identify key surrogate predictors of inflammatory and more destructive disease. It may now also be possible to look back and challenge (by using these new technologies) the key species associated within gingivitis and periodontitis. This is important as those species, now detectable using DNA probes, may have previously been difficult or impossible to culture.

The heterogeneity of the methods of analysis along with the experimental use of novel technologies has, so far, prevented a systematic review from being conducted into the diagnostic potential of biomarkers. The key to the success of future investigations is for researchers to work together with commonly agreed methods and protocols for biomarker analysis and reporting that allows comparability between studies and furthers the understanding of how periodontal inflammation develops, becomes established, resolves, and crucially, at what stage professional intervention is indicated.

Clinical measures remain important in evaluating and comparing the efficacy of plaque control interventions. In the established gingivitis model, the initial plaque control intervention of a powered toothbrush brought about expected resolution of mild gingival inflammation that was detectable clinically and by the shift in a broad bacterial profile that occurred towards those species historically associated with health. Improvements were observed following the subsequent intervention of scaling and prophylaxis with the hygiene phase having the greatest impact on clinically observed signs. Following evaluation of the sequential plaque control interventions, the intervention was applied to patients with gingival inflammation associated with oral lichen planus. The oral manifestations of lichen planus include an intense and refractory desquamative gingivitis which is frequently treated with topical immunosuppressive medication. Previous guidance lacked robust evidence, but suggested that some benefits may be elicited through effective plaque control (Erpenstein, 1985; Holmstrup *et al.*, 1990, Guiglia *et al.*, 2007; Lopez-Jornet and Camacho-Alonso, 2010a). An enhanced hygiene phase intervention was used to include additional interdental cleaning aids. It was subject to evaluation through clinical observations, patient-centred outcome

measures and subjected to a health economic evaluation. The overarching objective of the intervention was to address clinical signs of inflammation associated with oral lichen planus through self-performed plaque control.

The enhanced plaque control intervention brought about similar improvements in plaque scores to those observed in the original established gingivitis model as well as improving the extent and severity of the oro-mucosal disease. The effectiveness of plaque control in patients with oral lichen planus was unequivocal, suggesting that personalised plaque control programmes are important in managing the gingival manifestations of oral lichen planus. Plaque control should, therefore, form an important component of the initial treatment phase. It is also possible that reductions in inflammation may impact upon, and reduce the frequency, by which topical corticosteroids are subsequently required.

Host-derived biomarker analysis was also carried out for a small subset of the oral lichen planus population. Higher concentrations of IL-1β, MIP-1α, MIP-1β, RANTES, MMP-8 and MMP-9 were detectable in comparison to those observed in the established gingivitis model using the same methods of collection and analysis. Statistical comparisons were not performed but the concentrations were in the order of two-times those observed for IL-1β, MIP-1α, MIP-1β, RANTES, MMP-3, MMP-8 and MMP-9 in the gingivitis model. Possible explanations may be attributed to the immunological disregulation in the pathogenesis of oral lichen planus (Lodi *et al.*, 2005a). Given the chronic and refractory nature of oral lichen planus, it is not just pro-inflammatory but also regulatory cytokines that act on the gingival tissues (Yamamoto and Osaki, 1995). If the balance shifts towards those cytokines responsible for down-regulation then healing should occur, conversely increased levels are associated with inflammation. The local levels of cytokines observed support previous evidence that identified pro-inflammatory cytokine concentrations 2-3 times greater in oral lichen planus than in chronically inflamed tissue and 10-20 times those concentrations found in healthy gingiva (Yamamoto and Osaki, 1995). It is uncertain what impact topical immunosuppressant medication has on the local levels of inflammatory biomarkers, however it is reasonable to assume that higher concentrations might be observed in the untreated patient.

The inflammatory infiltrate observed in oral lichen planus lesions consists mainly of T-cells and in tissues exhibiting clinical signs of tissue destruction including atrophy and ulceration. It is also reasonable to assume that macrophages will be present in large numbers. IL-1β regulates the proliferation and differentiation of T-cells (Yamamoto and Osaki, 1995). The strong pro-inflammatory effect of IL-1β is likely to be partly responsible for the cellular infiltrate in oral lichen planus with higher levels associated with inflammation. Blocking of IL-1β activity has been recognised as a treatment for systemic autoimmune disease but not specifically oral lichen planus (Dinarello, 1994; Dinarello, 2011).

Expert clinical evaluation may be relatively straightforward to categorise the extent and severity of a disease into mild, moderate or severe or in the case of oral lichen planus reticular, atrophic or erosive. This clinical categorisation may, however, bear little or no resemblance to the symptoms that a patient experiences during the course of a chronic disease which are only really meaningful at an individual level. The chronicity of the disease is likely to play a key part in the impact that it has from the patients' perspective. The symptoms that are often recorded by clinicians relate to pain and discomfort, therefore treatment is initiated and based upon these symptoms alongside the presenting clinical picture.

Evaluation of the personalised intervention was developed from that used in the gingivitis model to include assessment of the impact that chronic oral inflammatory disease has on a patient's quality of life. The OHIP questionnaire aimed to further understand the frequency and nature of the impact of the disease and its management. When examining the baseline data it was clear that the subjects in the study reported impacts on their quality of life more frequently than healthy patients in a similar age cohort previously published (Slade and Spencer, 1994). Subjects in the intervention group reported significant improvements in their oral health related quality of life, but small improvements were also reported in OHIP and global change scores in both groups at 4-and 20-week follow up.

The impacts detected within the OHIP occurred in all domains, with the intervention reducing the frequency of these impacts compared to control. This was often in areas that would not usually be investigated as part of the routine clinical history. Those OHIP domains in which significant differences were

observed in the intervention group at both the 4- and 20-week follow up were in the functional limitation, psychological discomfort and physical disability domains. Analysis was not carried out at an individual question level and in-depth interviews were not carried out with subjects in this study. This may have provided further insight into the pre-existing effect that oral lichen planus has on a person's quality of life, and in what specific ways the intervention brought about these changes.

The initiation of lichen planus lesions has previously been reported to be co-incident with increases in stress and anxiety. It is possible that frequent observation through participating in a clinical trial may at some level, provide some psychological benefit. This observation is neither a placebo effect (this study was not placebo controlled) nor a result of the Hawthorne effect (the changes in behaviour as a result of being observed) (Roethlisberger and Dickson, 1939). Whilst potentially controversial, subjects may have placed value upon being monitored over time at more frequent intervals than they would ordinarily have as part of their conventional management pathway. Perhaps it was that participation itself provided a level of personal reassurance that their oral health was being closely monitored (Mahoney and Baker, 2002). This effect may be particularly important in a disease where patients are aware that there is a higher risk of malignant change.

The evaluation was then developed further through application of health economics. Economic evaluation provides supporting evidence to adopting an intervention if the benefits obtained by the patient exceed the costs of delivering it. Clearly if an intervention brings about clinical and/or symptomatic improvement but it is more expensive that alternative treatments clinical practice is unlikely to change. Cost-benefit and cost-effectiveness analyses are still relatively uncommon in clinical trials in dentistry and if resources are to be used effectively, evaluations of this kind should become an integral component of clinical trials. In this case the majority of subjects placed a higher value on the intervention than the actual costs of providing it, supporting its use in clinical practice.

On-going personalised supportive periodontal care should therefore be provided to patients with the gingival manifestations of oral lichen planus. The aim being to encourage patients to optimise plaque control, and minimise inflammation. The

clinical observations of the reductions in plaque levels and reduction in the activity of mucosal disease scores provide some evidence to justify managed care networks between oral medicine specialists, GDPs and hygienists. The demographics of these care pathways may change with the recent introduction of direct access to dental care professionals in the UK. This may allow referral of the patient directly from oral medicine specialist to dental hygienists or even oral health educators.

The challenge for this and other clinical research studies is in the translation and implementation of recommendations into everyday clinical practice. A number of pressures exist that encourage the *status quo* within general dental practice. Not all of these lie within the control of the individual practitioner but are subject to the pressures of regulation and working within much larger organisations, for example the NHS in the UK.

Conventional methods for disseminating research have focussed upon publication in esteemed academic journals. The interviews and focus groups conducted investigating this final translational step showed those who are engaged and at the forefront of primary care practice simply do not read, or even have access to, many of these academic publications. Consequently, the final step in the process of translating findings from biological and clinical research to the interface between clinician and patient may never be realised.

With the exception of peer reviewed publications and formally accredited programmes, few of the available sources of continuing professional development in the UK are quality assured in a rigorous way. They are also open to the personal and commercial biases of those organising and delivering the courses. Along with the exponential increase in continuing professional development, there is a risk that practitioners do not engage at all with journals, choosing instead to select sources that are easy to obtain certification from. Researchers must be cognisant of these changes and be adaptive to new methods and routes to disseminate their findings alongside more established routes of journal publication and presentation at specialist scientific conferences.

5.1 Conclusions

- Plaque control interventions comprising powered toothbrushing and professional prophylaxis are effective in reducing established gingivitis.
- The greatest improvements in plaque control were observed during the self performed hygiene phase of treatment.
- Sequential plaque control interventions brought about shifts in microbiological species towards those species associated with health.
- Transient increases in host-derived inflammatory changes were observed following sequential plaque control interventions.
- A personalised plaque control intervention was effective in reducing plaque, clinical signs of inflammation and bringing about improvements in quality of life for patients with gingival manifestations of oral lichen planus.
- A personalised plaque control programme for managing the gingival manifestations of oral lichen planus was cost-effective.
- Personalised plaque control should form part of the initial management phase for patients presenting with the gingival manifestations of oral lichen planus.
- Barriers exist to the successful translation and implementation of contemporary clinical research.
- Results from clinical research should continue to be published in peer reviewed publications but researchers should seek to disseminate findings using pathways that are more accessible to general dental practitoners.
- Clincal researchers should engage with postgraduate educators to ensure that research findings are disseminated to general dental practitioners more effectively.

5.2 Recommendations for further work

- Investigation of previously researched diseases should do so using pioneering methods and technologies which add to the understanding of the underlying pathology.
- Clinical intervention studies should, where possible, utilise self-reported outcome measures alongside clinical evaluations to understand the effectiveness of the intervention.
- Further qualitative work is required to further understand the impact of chronic oro-mucosal disease and its management from patient's perspectives. This may lead to the development of a shortened condition specific instrument that may be able to measure these constructs and inform clinical management.
- New interventions should be evaluated for their cost-effectiveness compared to standard treatments.
- New routes for the effective dissemination and translation of research findings should be explored and evaluated.

Appendix 1. The Oral Health Impact Profile

The conceptual dimensions and item numbers of the oral health impact profile. Items were administered in the original questionnaire format and the original order (1-49), responses were obtained on a Likert type scale from 5 options: never, hardly ever, occasionally, fairly often or very often.

Functional limitation

1. Difficulty chewing
2. Trouble pronouncing words
3. Noticed a tooth that doesn't look right
4. Appearance affected
5. Breath stale
6. Taste worse
7. Food catching
8. Digestion worse
17. Dentures not fitting

Physical Pain

9. Painful aching
10. Sore jaw
11. Headaches
12. Sensitive teeth
13. Toothache
14. Painful gums
15. Uncomfortable to eat
16. Sore spots
18. Discomfort

Psychological discomfort

19. Worried
20. Self-conscious
21. Miserable
22. Appearance
23. Tense

Physical Disability

24. Speech unclear
25. Others misunderstood
26. Less flavour in food
27. Unable to brush teeth
28. Avoid eating
29. Diet unsatisfactory
30. Unable to eat (dentures)
31. Avoid smiling
32. Interrupt meals

Psychological Disability

33. Sleep interrupted
34. Upset
35. Difficult to relax
36. Depressed
37. Concentration affected
38. Been embarrassed

Social Disability

39. Avoid going out
40. Less tolerant of others
41. Trouble getting on with others
42. Irritable with others
43. Difficulty in doing jobs

Handicap

44. Health worsened
45. Financial loss
46. Unable to enjoy people's company
47. Life unsatisfying
48. Unable to function
49. Unable to work

Appendix 2. Clinical Trial 1: Participant information sheet

The Newcastle upon Tyne Hospitals NHS
NHS Trust

Study Title

A Longitudinal Study to Characterize and Correlate Clinical, Microbial and Host Response Parameters in Subjects with Mild to Moderate Gingivitis.

[Lay title: Bacterial and inflammatory interactions in early gum disease]

Participant Information sheet

Invitation

You are being invited to take part in a research study. Before you decide whether or not to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read this information document carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part.

Thank you for reading this.

What is the purpose of the study?

The aim of this study is to look carefully at how the type of bacteria that cause gum disease (gingivitis) may change following the introduction of a tooth brushing programme and then after scaling the teeth. We also will attempt to identify whether there is any change in the way the gums respond to the bacteria that cause the disease.

Why have I been chosen?

You have been invited to take part in this study because you have some areas of gum inflammation in your mouth. Those sites where the gum is inflamed may be more difficult to clean and consequently the inflammation has developed as dental plaque builds up. Part of the study will involve you being asked to use a powered toothbrush with a view to changing the way you brush your teeth which may in itself have an impact on the gum condition. We will, however, be unable to recruit you to the study if you currently use a powered toothbrush.

Do I have to take part?

No, absolutely not. The decision is entirely up to you and, even if you decide to take part and then later wish to change your mind, you may do so without giving any reason.

What will happen to me if I take part?

The study lasts for a maximum of 10 weeks and altogether you will need to make 6 more visits to the Periodontology Department at the Dental Hospital. We will ask you to make sure that you brush your teeth between 2 and 6 hours before you attend all of these visits.

The first visit is called **Screening** when we shall undertake a clinical examination of your mouth, record some information about your general and dental health, weight, height, gender and race. Before we do so, we shall need to ask you to consent specifically to this initial examination before we can decide whether you are eligible to enter the study.

We shall then carry out a detailed inspection of your gums and this will involve the use of dental probes to make measurements of your gum condition. If these measurements show that you do not meet the criteria for taking part in the study then no further visits will be arranged. If you do have gingivitis then you will be given an appointment for the next visit and you will be asked to confirm your consent to take part in the study.

The next visit is called **Baseline** and this must be within 14 days of Screening. We will again record clinical measurements of your gum condition and also take samples of dental plaque and the fluid (called gingival crevicular fluid) that escapes from the margins of the gums when they are inflamed. The plaque will be removed from just above and just below the gum margin at 8 tooth surfaces using soft pieces of filter paper and a dental instrument. The gum fluid will also be collected using small strips of filter paper which will be tucked gently into the crevice between the gum and the teeth at the same sites from which the plaque is removed. At baseline we shall also give you a powered toothbrush and a toothpaste to use for the remainder of the study, as well as a diary to record how often you brush your teeth. We will also request that you don't use any other method for cleaning your teeth during the study.

We will see you again 2, 4, 6 and 8 weeks after baseline.

At the **week 2 visit** we shall measure the amount of plaque on your teeth and the redness of your gums using indices designed for this purpose. We will take gum fluid samples but not samples of dental plaque.

At the **week 4 visit** we shall repeat the indices recorded at week 2, and the clinical measurements taken at baseline as well as the plaque and gum fluid samples. At the end of this visit we shall provide a thorough scale and polish of all your teeth with a view to further improving the condition of your gums.

At the **week 6 visit** we shall repeat the stages of the week 2 visit: measure the amount of plaque on your teeth, redness of the gum and take gum fluid samples.

At the **week 8 visit** we shall repeat the stages of the week 4 visit but without the scale and polish of the teeth.

All the visits to the Hospital should take around 90-100 minutes and completion of the diary only about 2 minutes on each day.

At the end of the week 8 visit you will have completed all the stages of the study.

What do I have to do?

<u>In summary</u>, if you agree to take part in this study, we will ask you to:

- **visit the Department on 6 further occasions over a maximum of 10 weeks;**
- **undergo a series of clinical measurements to record the severity of your gum condition;**
- **allow us to remove samples of dental plaque and gum fluid;**
- **use the toothbrush and toothpaste we give you;**
- **have a thorough scale and polish;**
- **complete a diary to record your tooth brushing habits.**

What are the possible benefits of taking part?

There may be no benefit at all for you as a result of taking part. However, the regular monitoring of your gum condition together with better tooth brushing and following the instructions for keeping your teeth and gums clean will likely lead to a more healthy gum condition. The scale and polish of your teeth will also have a beneficial effect on your gum condition although in the longer term, any benefit will only be maintained by improved tooth brushing and regular visits to your dentist.

What options are there for my gum treatment?

For people with gingivitis, keeping the teeth and gums clean is essential and we will provide you with detailed information about how to do this irrespective of whether you enroll in the study. If we feel that your dentist might be able to contribute to the treatment of your gum condition then we will tell you and you will be able to discuss this with him/her when you next attend for a check-up.

What are the possible disadvantages of taking part?

The main disadvantage will be the number of visits you will need to make to the Hospital. None of the clinical measurements, the indices or taking samples of plaque and gum fluid are painful although sitting with your mouth open for long periods may be uncomfortable.

Will my dentist be informed of me taking part in the study?

We will ask you to consent to us writing to let your dentist know that you are participating in a clinical trial. We will let them know that you are participating because you have gingivitis and that as part of the study, we shall be providing some treatment for the condition. It is also possible that during the clinical examination of your mouth, we may discover some dental decay or other problems that your dentist will need to treat and it would obviously be helpful if we could let them know.

What if new information becomes available?

Sometimes, during the course of a research project, new information becomes available about the treatment, or in this case gum condition, that is being studied. If this happens, we will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research clinician will make arrangements for your care to continue.

Also, on receiving new information we might consider it to be in your best interests to withdraw you from the study. We will explain the reasons and arrange for your care to continue.'

What happens when the research stops?

When the study ends we would like to write to your dentist to let him/her know. Should we feel that your dentist needs to provide you with any specific gum treatment on a long-term basis then we shall let him/her know and we shall send you a copy of the letter.
It is absolutely crucial that you appreciate that all dental treatment other than that for your gum condition will continue to be provided by your dentist. This includes regular dental check-ups and any fillings that you may require.

What if something goes wrong?

If you are injured as a result of taking part in this trial, Philips Oral Healthcare, without legal commitment, will compensate you without you having to prove that it is at fault. The Sponsor will not compensate you, however, where such injury results from any procedure carried out which is not in accordance with the protocol for the study. Your right at law to claim compensation for injury where you can prove negligence is not affected.

Will my taking part be kept confidential?

If you consent to take part in the research the clinical measurements that we make will be used by Philips Oral Healthcare for purposes of analyzing the results. They may also be looked at by people from regulatory authorities to check that the study is being carried out correctly. **Your name, however, will not be disclosed outside the Dental School and Hospital**.

All of the data that we record will be entered into an electronic Web-based data system which has ID codes and password protection. Your information that goes into the system will be coded by a number; **the link between this number and your name will only be kept at Newcastle University**. Samples of bacteria and gum fluid will be sent to laboratories in the United States for analysis and these will be labeled only with your unique study number and not your name.

What will happen to the results of this study?

It is likely that the results from the study will be presented to a major dental research meeting although your name will not at any time be disclosed. The results will also be written up as a research paper and published in a specialist dental journal that deals entirely with gum conditions and diseases.

Who is organizing and funding the research?

The Sponsor is Philips Oral Healthcare who is based in the United States. They have provided funds to Newcastle University for the research team to run the study although the researchers themselves receive no payments.

Compensation for your time and effort

Philips Oral healthcare, the sponsor of the study, has agreed to provide compensation in recognition of the time you spend attending the visits for this project. For attending each of the next 6 visits you will receive a gift voucher worth £10 and then a further gift voucher for £20 if you complete the project. That is a total of vouchers worth £80. In addition, you will be provided with a new Sonicare FlexCare powered toothbrush for your use at home.

Who has reviewed the study?

The ethical issues associated with this study have been reviewed by a Research Ethics Committee (REC) and a favourable opinion has been given. If there are any changes to the way the study is run the research team must inform the REC and seek further opinion.

Contacts for further information

There is a chance that you may need to contact one of the research clinicians (dentists) during the trial, for example to change appointment times or should you have a problem with the toothbrush. The contact information below will enable you to contact a member of the research team:

Main contact:

Mrs Moira Swan

Room 5.010; Tel: (0191) 2228188; Email: moira.swan@ncl.ac.uk

Additional contacts:

Professor Peter Heasman	Dr Giles McCracken	Mr Simon Stone
Room 5.017	Room 5.011	Room 5.014
Tel: (0191) 222 7824	Tel: (0191) 222 8194	Tel: (0191) 222 8515
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p.a.heasman@ncl.ac.uk	g.i.mccracken@ncl.ac.uk	simon.stone@ncl.ac.uk

Appendix 3. Clinical Trial 1: Consent form

The Newcastle upon Tyne Hospitals NHS
NHS Trust

A Longitudinal Study to Characterize and Correlate Clinical, Microbial and Host Response Parameters in Subjects with Mild to Moderate Gingivitis.

[Lay title: Bacterial and inflammatory interactions in early gum disease]

Consent Form

Part 1

Please read this form carefully and initial the boxes next to the statements if you agree with them.

I acknowledge that I have read the information sheet, which outlines the clinical study. I have also received verbal information regarding the requirements and commitments for participants in the study and I have had the opportunity to ask questions, either to the principal investigator or to another member of the research team. ☐

I have had sufficient opportunity to reflect fully on the explanation of the trial. ☐

I consent to my participation in a **Screening examination** to see whether I am eligible to take part in the study. I acknowledge that a copy of this consent form and information sheet will be provided to me on request. ☐

_____	_____
Name of subject	Date
_____	_____
Signature of subject	Date
_____	_____
Signature of investigator	Date

Consent Form

Part 2

I understand that the screening examination has shown that I am eligible to take part in the study. ☐

I appreciate that participation is entirely voluntary and that I am able to discontinue at any time and without giving reason. ☐

I consent to my dentist being contacted to inform him/her that I am taking part in the study and to let him/her know that I may need other dental treatment in due course. ☐

_____	_____
Name of subject	Date
_____	_____
Signature of subject	Date
_____	_____
Signature of investigator	Date

Appendix 4. Clinical Trial 2: Participant information sheet

Confidential OHI.DG.01

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Newcastle University

Study Title

A tailored oral health intervention for patients with desquamative gingivitis

Participant Information Sheet

Invitation

You are being invited to take part in a clinical research study, before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information or you have problems reading then we can assist you with this. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

This study aims to evaluate the effect of good oral hygiene practice, specifically tooth-brushing and inter-dental cleaning on patients with sore gums and mouths. The effectiveness will be measured by completing questionnaires that measure how problems with your mouth affect your quality of life. In addition to this we will identify if there is any clinical changes in your mouth.

Why have I been chosen?

You have been selected because you have a collection of clinical signs in your mouth known as 'desquamative gingivitis,' this may caused by a number of diseases, the most common being 'oral lichen planus.' The most common signs are redness or blistering of the gums which can be very painful. These sore areas are where the gum is inflamed may be more difficult to clean and dental plaque may build up. Part of this study may involve you changing the way in which you clean your teeth and mouth which may itself have an impact on the soreness of your mouth. This study has been designed to so it does not delay or affect any treatment, should you require it, in the future.

Do I have to take part?

No, absolutely not. The decision is entirely up to you and, even if you decide to take part and then later wish to change your mind, you may do so without giving any reason. A decision to withdraw or a decision not to take part, will not affect the standard of care you receive or your treatment in the future. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form.

What will happen to me if I take part?

Participation in the study will involve **three visits to the Dental Hospital of approximately 30 minutes in duration**. We will ask you to brush your teeth between 2 and 6 hours before you attend all of these visits. If you choose to participate; **you will be randomly allocated into one of two groups**, just like tossing a coin, this will be done at random and a sealed envelope with your study number will be opened revealing which group you will be placed in.

In the **first group** we will monitor how the condition of your mouth changes and impacts on your life over the next 20 weeks. We will ask you to complete a questionnaire, we will then carry out a detailed inspection of your mouth and record measurements of how extensive the sore areas in your mouth are and how much dental plaque you have. If you are selected for this group we will ask you to continue to clean your teeth and mouth as normal.

The questionnaire will relate to how the condition of your mouth affects your quality of life, in other words to what extent does it interfere with your daily life and has 49 statements, you will be asked to choose how often each of the statements best applies to you. We will also ask a further question about how painful your mouth is at the moment.

After the first visit we will also ask you if you think that your condition has improved or worsened. At each visit we will also take some photographs of the inside of your mouth using a digital camera, your face will not be visible on these images and it will not be possible to identify you from them.

The **second group** will have similar information recorded as the first, requiring the same number of visits and time, the only difference is this group of participants will be provided with an oral hygiene programme to follow throughout the duration of the study, this will include the use of a powered toothbrush and other aids for cleaning in between your teeth. We will provide you with these.

At subsequent visits we will record the same information to see how the condition of your mouth changes over time.

We are also interested in the cost effectiveness of providing this treatment to other patients. At the end of the study we will ask you to complete a short questionnaire which will record out of pocket payments and lost work time during the trial period. In addition those in the oral hygiene programme group will be asked how much they would have been willing to pay to purchase the intervention. Please note this is for our information only and **there is no cost to participate in this study**.

For a small number of participants we will take a small saliva sample and some fluid from the gum using filter paper. The samples will be used for biological analysis and once analysed will not be stored for future use. They will be used solely to determine inflammatory markers of disease and not for human DNA analysis.

What do I have to do?

In summary, if you agree to take part in the study we will ask you to:

- Visit the Dental Hospital on 3 further occasions over a period of 20 weeks;
- Complete a questionnaire at each visit to record how the condition of your mouth affects your quality of life;
- Undergo a series of clinical measurements to record the severity of the condition of your mouth;
- Use the tooth brush and inter dental cleaning aids we give you;
- Have a professional polish of your teeth.

What are the possible benefits of taking part?

There may be no benefit at all for you as a result of taking part. We hope that your involvement will help us to improve our understanding of the impact of desquamative gingivitis on quality of life and therefore lead to a more appropriate, targeted approach to treatment. This should ultimately result in you and others like you receiving an improved level of care in the future.

Regular monitoring of the condition of your mouth together with better tooth brushing and following the instructions for keeping your teeth and gums clean will likely lead to a more healthy gum condition.

The powered toothbrush, a Philips Sonicare Flexcare will be yours to keep at the end of the study for those allocated into the non-intervention group this will be provided at the final visit.

Reasonable travel expenses will be reimbursed for appointments at the Dental Hospital that are in addition to your normal visits to the hospital, these will be made upon completion of the study and upon production of appropriate travel receipts.

What are the possible disadvantages of taking part?

The main disadvantage will be the number of visits you will need to make to the Hospital. None of the clinical measurements are painful.

What if new information becomes available?

Sometimes, during the course of a research project, new information becomes available about the treatment or condition that is being studied. If this happens we will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research clinician will make arrangements for your care to continue.

Will your participation in this study be kept confidential?

Yes. All information that is collected about you during the course of the research will be kept strictly confidential; any record forms will be labeled with your study number and not your name. The information we record may be looked at by people from regulatory authorities to check that the study is being carried out correctly. Your name however will not be disclosed outside the Dental School and Hospital.

What if there is a problem or something goes wrong?

If you have any concerns that arise as a result of taking part in this study, you can contact the study co-ordinator on 0191 2226378, they will do their best to answer your questions. If you remain unhappy and wish to complain formally, we will provide information about the National Health Service Complaints Procedure.

If something goes wrong through someone's negligence, you may have grounds to seek legal advice. The Newcastle upon Tyne NHS Foundation Trust is the sponsor for the study and accepts responsibility for the study's conduct.

What will happen to the results of this study?

It is likely that the results from this study will be presented to a major dental research meeting although your name will not at any time be disclosed. The results will also be written up as a research paper and published in a specialist dental journal and as part of a research degree.

Who is organising the study?

The research is being carried out by Mr. Simon Stone, a dentist who is undertaking research with a group of patients who have similar problems with their mouths to you. He will be using the results of this study as part of his research degree (PhD), this research is funded by the School of Dental Sciences at Newcastle University, the funding covers only the costs of the necessary expenses to run the study.

Who has reviewed the study?

The ethical issues associated with this study have been reviewed by a Research Ethics Committee (REC) and a favourable opinion has been given. If there are any changes to the way the study is run the research team must inform the REC and seek further opinion.

What will happen at the end of the study?

At the end of the study you will continue with your standard treatment at the Dental Hospital. You are free to continue using the toothbrush and other cleaning methods we have shown you throughout the course of this study, for those not in the intervention group, you will be provided with the same powered toothbrush at the end of the study.

Contacts for further information

There is a chance that you may need to contact one of the research clinicians (dentists) during the trial, for example to change appointment times or should you have any further questions. The contact information below will enable you to contact a member of the research team:

Main contact

Mr. Simon Stone

Room 5.012; Tel: (0191) 222 8515

Additional contacts

Dr Giles McCracken
Room 5.011
Tel: (0191) 222 8194

Professor Peter Heasman
Room 5.017
Tel: (0191) 222 7824

Appendix 5. Clinical Trial 2: Consent Form

Confidential OHI.DG.01

The Newcastle upon Tyne Hospitals NHS Foundation Trust

Newcastle University

Study Title

A tailored oral health intervention for patients with desquamative gingivitis

Consent form	**Please initial box**
I confirm that I have read and understand the information sheet for this study and I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals involved in the study or from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
I consent for clinical photographic images of my mouth to be taken and for the use of these images in clinical research, and for use in educational publications, journals, textbooks and electronic publication. The images may also be used for teaching of professional staff and dental students and for medical records. These images will not be identifiable to me.	☐
I consent to samples of saliva and fluid from my gums (gingival crevicular fluid) being taken and used for biological analysis, I understand these samples once collected will not be identifiable to me. The samples once stored, will **not** be used for human DNA analysis.	☐
I consent to Newcastle University contacting my General Dental Practitioner informing them of my participation in this study (optional).	☐

I agree to take part in the above study.

Name of Patient	Date	Signature
Researcher	Date	Signature

Version 2 27/09/2011

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