Analysis of biological pathways, associated with orthodontic force, using multiplex arrays.

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A thesis submitted to the University of Birmingham for the degree of

MASTER OF PHILOSOPHY

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October 2010

DEDICATION

To my husband Paul without whose help and support I would not be writing this today.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Professor I.L.C Chapple and Dr W.P Rock for their help and guidance through this study.

I would also like to thank Mr P.J Turner and Mrs S.J Church for their assistance in making the clinical aspect of this thesis possible and Dr M Grant for helping me with the laboratory side of this study and the writing of this thesis.

ABSTRACT

Objective

The objective of the proposed study was to investigate tissue changes induced by orthodontic forces at both tension and compression sites, with a view to better understanding the biological processes that lead to differing rates of tooth movement and adverse events.

Methods

Gingival Crevicular Fluid (GCF), was collected from mesiobuccal and distopalatal locations on maxillary canine and second molar teeth using Periopaper™ strips for 30s from volunteers (n=21). Samples were taken at: baseline, before appliance placement and tooth extraction (B); three months into orthodontic treatment (T1); at four hours (T2); 1 week (T3); and 6 weeks (T4) after a distalising force had been applied to the maxillary canine teeth. At each time point detailed periodontal health measures and plaque scores were recorded, as well as impressions of the teeth. Study casts were used to measure canine movement. GCF volumes were recorded and proteins eluted into sterile water and stored at -80°C. Cytokines (GM-CSF, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10 and TNFα), tissue (MMP-9, TIMP-1 and 2) and bone metabolism (RANKL and OPG) biomarkers were measured using muliplex protein arrays on a Luminex 200™ machine.

Data analysis

Data were analysed using a Prism computer softwear programme.

Results

Tension sites at the maxillary canines showed increases in IL-1β, IL-8, TNFα, MMP-9 and TIMPs 1 and 2 across time points T2-T4, whereas the compression sites for these teeth showed increases in IL-1β and IL-8 only at T2, MMP-9 at T3 and T4 and RANKL at T4. GCF volume increased from T2 to T4 but plaque scores or bleeding on probing did not increase over this time. Increases in the levels of biomarkers were more consistently detected at sites of tension than of compression. Other biomarkers were below assay detection limits.

Conclusions

There were significant changes in the profile of several biomarkers at the various time points. These were consistant with tissue remodelling. Although differences were seen between tension and compression sites, none of these were statistically significant.

Increases in TNFα, GM-CSF, IL-1β and IL-8 correlated with the rate of tooth movement. MMP-9, TIMP-1 and TIMP-2 correlated with rate of movement 4 hours after application of a distalising force.

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Chapter 1

Introduction and Background

1.1 Introduction

Application of an appropriate force to a tooth results in tooth movement. Orthodontic therapy is based on this principle and relies upon remodelling of the periodontal ligament, gingival soft tissue and alveolar bone (periodontium) in order to allow tooth movement, during which tissue is removed ahead of the tooth and deposited behind.

The periodontium consists largely of connective tissue. For optimal rates of tooth movement the biological reaction generated in response to orthodontic force must be optimised to prevent iatrogenic and degenerative changes including resorption of root dentine.

Many factors influence the course of orthodontic treatment, including patient compliance and individual biological responses to the application of orthodontic force (Reitan, 1954). Gingival crevicular fluid (GCF) is a serum transudate. During its passage from the vascular system, through the periodontal tissues and into the gingival crevice, various substances (microbial or host-origin) are incorporated which reflect the biology and physiology of the local tissues (Last et al., 1988). Studies have used gingival crevicular fluid to assess changes within the periodontium and have related those changes to tooth movement. However the majority of studies have assessed individual biomarkers, which are representative of only a single biological pathway. Waddington and Embery (2001) concluded that "It may well be that a package of biomarkers may provide a more complete answer".

Protein/cytokine arrays provide a contemporary technology necessary to simultaneously profile different pathways, including inflammatory cytokine (e.g. IL-1, TNFα) pathways and bone metabolic pathways involving matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). MMPs are zinc-dependent proteases with the capacity to degrade extracellular matrix proteins (e.g. collagen, elastin). MMP-2 and 9 and TIMP-1 and 2 are thought to be important in bone metabolism (NIH, 2006). In the present study panels of cytokines, tissue and bone metabolites were quantified in GCF using protein arrays, to profile the biological response to orthodontic force.

1.2 Background

1.2.1 Remodelling of the periodontium

Orthodontic tooth movement requires remodelling of the periodontal tissues, with tissue removal ahead of the tooth and the deposition and re-establishment of tissue architecture behind. Orthodontic therapy is dependent on this remodelling.

When similar force systems are set up in different individuals the rate of tooth movement varies due to differences in bone density, connective tissues, and cellular responses within the periodontal ligament.

Whilst the response of individual teeth to orthodontic force is important, there is little research available to enable the orthodontist to guage ideal force levels and thus achieve optimal rates of tooth movement. It is known that subjects vary in their responses to orthodontic forces irrespective of age (Reitan, 1954), but it is not known how to predict this.

1.2.2 Bone remodelling

Bone structure is intimately related to function; an outer layer of dense cortical bone surrounds a core of less dense trabecular bone. The cells predominantly responsible for the turnover of bone matrix are osteoblasts and osteoclasts.

1.2.2.1 The bone remodelling cycle

The initial steps of bone remodelling involve the differentiation and activation of osteoclast precursors to form osteoclasts, which then begin the process of bone resorption. Resorption is followed by bone formation, the number of sites entering a phase of bone formation combined with the rate of resorption will determine overall turnover (Ericksen et al., 1986; Charles et al., 1987).

As mentioned previously, the process of bone resorption and formation are interlinked. Details of the linking mechanisms are unknown, although transforming growth factor (TGF)-ß, insulin-like growth factor (IGF) and plasminogen activators have all been suggested (Martin and Ng, 1994).

The osteoclast is the primary resorbing cell. Initially there is recruitment and dissemination of osteoclast progenitors. Such cells are haemopoietic in origin and are related to the monocyte-macrophage lineage. Differentiation into osteoclasts is thought to involve cell-to-cell interaction with osteoblast stromal cells (Suda et al., 1996).

The bone surface is prepared by the removal of the surface un-mineralised osteoid by the osteoblasts which line it and produce many proteolytic enzymes including MMPs, collagenase and gelatinase. Osteoclasts recognise extracellular bone matrix proteins such as osteopontin through cell surface integrin adhesion molecules.

The osteoclast then becomes activated at the surface of the mineralised bone. Such activation is thought to be mediated through the osteoblast via local factors or cell-to-cell contacts (Fuller et al., 1991).

Osteoclasts resorb the bone surface by producing hydrogen ions, which change the pH balance and dissolve the inorganic elements. Proteolytic enzymes then degrade the organic components. The organic matrix is composed mainly of collagen type I (90% of the protein in bone) and is degraded by two main types of enzyme, the lysosomal cysteine proteinases cathepsin B, L, and K together with the matrix metalloproteinases; collagenase and gelatinase B.

The cessation of osteoclast activity results from apoptosis (programmed cell death), which suggests that the regulation of osteoclast life span may be another important determinant of bone metabolism.

1.2.2.2 Bone deposition

Around nine days following the onset of resorption, when the osteoclast has eroded the bone surface to a maximum depth, a reversal process begins. Osteoclastic activity is reduced by apoptosis. Increased calcium levels within the sub-cellular space inhibit further resorption whilst substances released from the bone matrix during resorption initiate osteoclast deactivation.

As with resorption, bone deposition is the end point of a complex cascade of events involving proliferation of primitive mesenchymal cells, formation of preosteoblasts, matrix formation and mineralization. Osteoblasts converge onto the base of the resorption cavity to form osteoid. Cells are attracted towards the resorptive defect by local factors including Transforming Growth Factor β (TGF-β) and the exposed type I collagen. Local proliferation of osteoblast precursors is influenced by TGF-β ,Insulin-Like Growth Factor (IGF) I and II, Fibroblast Growth Factor (FGF) and Platelet Derived Growth Factor (PDGF).

Differentiation of cells into mature osteoid-producing cells is mediated by IGF-I and BMP-2. Osteoid begins to mineralise 13 days later at a rate of approximately 1μm per day (Ericksen et al., 1986). Bone formation continues until the cavity is filled, over 120-180 days.

1.2.3 Theory of tooth movement.

The first theory was described by Burstone (1962) who suggested that there were 3 phases of tooth movement: an initial phase, a lag phase, and a post lag phase.

In the initial phase there is rapid movement after the application of force largely attributable to displacement of the tooth in its socket. After the initial phase, there is a lag period due to hyalinization in areas of compression. No further tooth movement occurs until cells complete the removal of necrotic tissue. The third phase of tooth movement follows the lag period, during which the rate of movement gradually or suddenly increases (Burstone, 1962).

Other studies have proposed a time/ displacement model for tooth movement (Pilon et al., 1996, Vas Leeuwen et al., 1999). These studies divided the curve representing tooth movement into 4 phases. The first phase lasts 24 hours to 2 days and represents initial movement of the tooth inside its bony socket (Burstone's initial phase). This is followed by a second phase, when tooth movement stops for 20-30 days (Burstone's lag phase). After removal of necrotic tissue tooth movement is accelerated in the third phase (Burstone's post lag phase) and continues into the fourth phase.

Neither hypothesis provides conclusive evidence to explain the detailed nature of the biological mechanism of tooth movement. Other studies in the 20th century and the early 21st century demonstrated that many cellular and tissue reactions start in the initial phase of tooth movement, immediately after force application. Because of the compression and stretch of fibres and cells in the PDL, the complex process of recruitment of osteoclast and osteoblast progenitors, as well as extravasation and

chemoattraction of inflammatory cells begins. Blood flow disruption due to distortion of the PDL fibres leads to the development of hyalinized areas and the arrest of tooth movement, which can last 4-20 days. Only removal of necrotic tissue and bone resorption from adjacent marrow spaces (indirect resorption) and from the direction of the viable PDL (undermining resorption) allow the resumption of tooth movement. This comprehensive process requires the recruitment of phagocytic cells such as macrophages, foreign body giant cells, and osteoclasts from adjacent undamaged areas of the PDL and alveolar bone marrow cavities. These cells remove necrotic tissues from compressed PDL sites and adjacent alveolar bone. In areas of PDL tension, quiescent osteoblasts are enlarged and start producing new osteoid. Osteoblast progenitors are recruited from the population of fibroblast-like cells (pericytes) around PDL capillaries, which proliferate and migrate toward the alveolar bone surface, along the stretched Sharpey's fibers. Simultaneously, PDL fibroblasts in tension zones begin multiplying and remodelling their surrounding matrix.

The third and fourth phases of orthodontic tooth movement, also known as the acceleration and linear phases, respectively, start about 40 days after initial force application. The pressure sides of teeth exhibit collagen fibres without proper orientation. Here, irregular bone surfaces are found, indicating direct or frontal resorption.

The development and removal of necrotic tissue is a continuous process during tooth displacement, rather than a single event (von Böhl et al.,2004 a and b). This conclusion is supported by one of Melsen's hypotheses that "indirect bone resorption at the pressure side is not a reaction to force but an attempt to remove ischemic bone

lying adjacent to the hyalinised tissue. The subsequent direct bone resorption could be considered part of the remodelling process."The tension sides in the third and fourth phases clearly show bone deposition, as evidenced by alkaline phosphatase positive osteoblastic cells" (Melsen, 1999).

Teeth subjected to high forces show hyalinization more often than teeth experiencing light forces. Thus, development of hyalinization zones has a definite relationship to the force magnitude, but it was found to have no relationship to the rate of tooth movement. Once tooth movement has started after the second (arrest) phase, bone remodelling takes place at a certain rate, independent of force magnitude (Von Bohl et al., 2004). These findings agree with those of Owman-Moll et al. (1996) and Van Leeuwen et al. (1999), who also reported the location of hyalinization was found mostly buccal or lingual to the mesio-distal plane. Studies have also shown that a lag period exists between an abrupt change in mechanical loading and the attainment of maximal response (Jaworski et al., 1980).

1.2.4 Remodelling of the periodontal ligament

1.2.4.1 Areas of compression

Compression of the periodontium produces narrowing of the periodontal ligament and vascular restriction. This can lead to ischaemia, necrosis and vessel degeneration. A high enough force can lead to hyalinisation or sterile necrosis. However if the periodontal ligament can be maintained despite the reduction in blood supply, cellular activity increases with differentiation of monocytes into fibroblasts and osteoclasts. These two cell types are responsible for remodelling of the soft and hard tissues. The breakdown products of tissue turnover may be detected in GCF (Last et al., 1988).

Areas of hyalinisation are devoid of blood supply, so that the host is unable to recruit a cellular inflammatory response and resorption cannot occur. However, orthodontic force is distributed unevenly within the periodontal ligament, there are adjacent areas of normal periodontium and these areas contribute to remodelling of the hyalinised areas as osteocytes and vascular elements penetrate hyalinised tissue, whilst the alveolar bone in this area is removed by underlying resorption. (Rygh et al., 1973).

Removal of bone from areas of compression is not a simple or continuous process. Following application of orthodontic force, small bony changes occur within the first three days, followed by a wave of resorption lasting one week, which is then followed by bony deposition (King et al., 1992). Bone remodelling can continue for some time even after the orthodontic force has been removed (King et al., 1992). In order to investigate host response to orthodontic force, a period of time should separate force application and assessment of the response to that force.

Osteoclasts within the resorptive area arise from two sources, some are derived locally and arrive within hours of the initiation of orthodontic force (Reitan, 1951), some arrive two days later and arise from the haematogenous cell population (Roberts and Ferguson, 1989). Although osteoclasts are the primary resorptive cells, osteoblasts possess receptors for the chemical signal of resorption namely; parathyroid hormone, eicosanoids (e.g. Prostaglandin E_2) and various cytokines (Meghji, 1992).

Bone resorption is undertaken by organic acids, lysosomal enzymes, matrix metalloproteinases (MMPs) and serine proteases (Keeling et al., 1993). Thus the inorganic components are dissolved and the organic matrix digested in a process called frontal resorption**.**

1.2.4.2 Areas of tension

Fibres of the periodontal ligament stretch in areas of tension. This is associated with vascular dilatation and an increase in local vascular activity (Rygh et al., 1986). In areas under tension the volume of collagen reduces in the first three days whilst vascularisation increases (Crumley, 1964). Mechanical stress leads to the production of collagenase enzymes (MMPs) produced by macrophages and fibroblasts (Sandy, 1992). Collagen fibres within the periodontal ligament exist in coiled forms. When tooth movement exceeds the intrinsic fibre length, new fibres must be synthesised and incorporated into the ligament proper.

Osteoblasts are generated from progenitor cells locally within the periodontium (Roberts and Chase, 1981). Following vascular dilatation, an area of intense increased vascular activity develops. Osteoblasts form sheets of densely packed cells on the bone surface and secrete the extracellular organic matrix of bone; components include type I collagen, osteocalcin, osteoponin and osteonectins. Cytokines, proteoglycans and growth regulating factors are also released. Osteoid deposition begins some two days following the application of orthodontic force and shortly afterwards mineralisation occurs within the deeper osteoid layers, although the surface layers remain un-calcified (Rygh et al., 1986).

1.2.5 Control of orthodontic tooth movement

There is still much to be discovered about the biological basis of orthodontics. Four theories have been proposed:

- The biomechanical theory
- The pressure/tension theory
- The piezoelectric theory
- The hydrodynamic theory

The hydrodynamic theory will not be discussed in this thesis.

1.2.5.1 Biomechanical theory

The biomechanical theory is based on mechanical distortion of cell membranes and resultant activation of phospholipase A_2 (Sandy et al., 1993). Phospholipase A_2 initiates arachidonic acid metabolism, which in turn activates the lipoxygenase pathway (leukotrienes and HETE) and the cyclo-oxygenase pathway (prostoglandins). There is clinical evidence that both leukotrienes and prostoglandins are linked to tooth movement (Yamasaki et al., 1989; Mohammed et al., 1989). Prostaglandins feedback to G protein coupled receptors and stimulate second messenger cascades, resulting in a cellular response that causes bone removal at compression sites and deposition at tension sites.

1.2.5.2 Pressure-tension theory

The pressure-tension theory relates changes in periodontal ligament blood flow, following the application of pressure, to the release of chemical messengers that effect cellular responses. Tension and compression within the periodontal ligament result in a gradual relocation of periodontal tissue fluids. Blood flow is reduced in areas of compression, but maintained or increased in areas of tension. Changes in blood flow may be in the order of 50 percent, with vascular endothelial junctions being important pathways for fluid transport (Tang et al., 1993). Reduction in blood flow lowers oxygen tension and a mild inflammatory reaction develops (Tuncay et al., 1994). Recruitment of different cell types produces a local environment rich in biologically active substances, further modulated by systemic factors; parathyroid hormone, calcitonin and vitamin D, that can also influence the production of cytokines and prostaglandins (Dewhurst et al., 1987).

Fibroblasts within the periodontal ligament are involved in collagen turnover and mediate biochemical transfer of the mechanical force applied orthodontically to the teeth and to the surrounding tissues, including the alveolus. Biochemical signals involved in this process include prostaglandins, neurotransmitters, cytokines and interleukins (Ten Cate et al., 1976). Intracellular events including the elevation of intracellular calcium follow the direct stimulation of isolated fibroblasts by hydraulic pressure (Nakago-Matsuo et al., 1996). Raised levels of intracellular calcium cause cellular responses, including proliferation, differentiation and the secretion of additional biological signals (Berridge, 1993).

1.2.5.3 Piezo-electric theory

Piezoelectric theory relates the generation of electrical charge following the distortion of bone matrix. Applied stress results in a reorientation of matrix proteoglycans which induce changes in cell membrane polarity and activation of membrane enzymes (Grimm, 1972). This leads to cell-membrane interactions and the opening or closing of stress-related ion channels (Davidovich, 1991). McDonald and Yettram (1995) examined the effects of mechanically loading bone on the ionic distribution in osteocytes. They found that when an external force was applied to osteocytes there was a change in the sodium ion and a great change in potassium ion distribution within the cells , but no change in chloride ions. A similarity between the pattern of distribution of strain energy density and the ions within the osteocytes was also seen.

Areas of compression become electronegatively charged whilst areas of tension become electropositively charged. The end result is that bone is resorbed and deposited simultaneously, the turnover equilibrium being shifted to favour one or the other at different times. For remodelling to occur the strain level should exceed a value of 0.02N (Frost, 1983). Finite element analysis has shown that this level can be exceeded in the periodontal ligament (Middleton et al., 1996). The periodontal ligament appears to experience higher strains than adjacent bone and dentine. Middleton et al. (1996) suggested that bone may not experience sufficient strain to begin remodelling during normal orthodontic loading.

1.2.6 Effect of orthodontic forces on the periodontum

1.2.6.1 The gingival tissues

The perceived benefits resulting from orthodontic treatment must be weighed against the potential harm, including the development of plaque-induced gingivitis and gingival hyperplasia. These conditions may be reversible alter appliance removal and appropriate oral hygiene measures (Zachrisson and Zachrisson, 1972) or they may require surgical intervention.

Pseudopocketing of the periodontium may arise because of an increase in gingival margin height relative to the cemento-enamel junction (CEJ). It is therefore reversible, maximum reduction in pocket depth occurring within one month following appliance removal and institution of oral hygiene methods. Further reductions continue for up to four months post treatment. There is no significant difference in pocket depth between test and control teeth after two years in healthy adolescent patients (Zachrison and Alnaes, 1973). Therefore, orthodontic treatment does not seem to have any permanently damaging effects upon the gingival tissues in healthy adolescent patients.

1.2.6.2 Connective tissue attachments

There is individual variation in the attachment losses experienced by different subjects. Boyd et al. (1989) found no clinically significant attachment losses in adolescents and adults following orthodontic treatment, if normal periodontal tissues were present before and throughout treatment (Boyd et al., 1989). However, Zachrisson and Alnaes (1973) found a statistically significant difference in mean

attachment loss between an orthodontically treated group (0.41mm) and an untreated control group (0.11mm) with certain individuals losing 2mm of attachment at some sites (Zachrisson and Alnaes 1973). Similarly Hamp et al. (1982) found a 0.28mm bone loss around bonded teeth during treatment compared to 0.22mm in the control group over the same period. Several explanations may account for the control/treatment group variation in these studies. It is difficult to evaluate attachment loss with accuracy as a result of differing methodologies, oral hygiene levels, tissue inflammation, probing force, probing angle, probe design, appliance types, differing gingival-bracket distances, position of the probe around the gingival margin, and visibility of the probe's scale to the operator.

The amount of force used during tooth movement is thought to influence loss of alveolar bone during orthodontic treatment (Reitan 1974). In the absence of plaque and with orthodontic forces at physiological levels, bone loss should not occur as a result of orthodontic movement (Eliasson et al., 1982).

It is incorrect to assume that "physiological" force levels are those that would not produce a pathological reaction in all cases, since the threshold values for bone remodelling vary between individuals. As the pathological reaction of greatest concern is root resorption one does not know if this sequel is present unless radiographs are taken and it may be that the incidence of root resorption is higher than that reported. Other influences on crestal bone include closure of extraction sites and the effects of calculus and plaque when they are moved from a supragingival environment following tooth up righting (Zachrisson and Zachrisson, 1972).

Patient age during orthodontic treatment also influences the potential risk of alveolar bone loss. Adults may experience greater amounts of bone loss than adolescents, since the experience of periodontal disease is higher in this group (Reitan, 1954; Van Der Velden, 1984). Also, it is rare to find significant alveolar bone loss in the early teenage years. Zacharrisson and Alnaes (1974) looked at 51 adolescents with a Class II division I malocclusion treated following extraction of upper first premolars and compared them to an untreated group. Standardised radiographs demonstrated a CEJ to alveolar crest distance of 1.1mm in the treatment group compared with 0.88mm in the control, so that orthodontic treatment was associated with 0.22mm additional bone loss (Zachrisson and Alnaes, 1974).

Orthodontic treatment at that time (1970s) involved the use of bands cemented to the teeth to carry the bracket into which the orthodontic wires were fitted. Often such bands could only be placed subgingivally in order to obtain a correct position of the bracket on the tooth and this may have produced bone loss. Modern appliances use the acid etch technique to attach brackets to the tooth, brackets are much smaller than bands and subgingival positioning is thus far less common. Also brackets are usually placed only on the buccal surfaces of the teeth.

1.2.6.3 Periodontal health monitoring in the orthodontic patient

Gingival and periodontal diseases are largely diagnosed by visual criteria and physical examination of the periodontal tissues using probes of various designs. During a periodontal examination the clinician looks for several specific indicative factors, including;

- Tissue colour and contour
- Presence of bleeding on probing
- Extent of gingival recession
- Probing pocket depth and attachment levels
- Presence of suppuration
- Degree of tooth mobility
- Presence, position, number and size of tooth furcations
- Radiographic evidence of bone loss

These methods are largely qualitative and are dependent on the preferences and estimations of the individual clinician, leading to possible variation in results, unless the clinician is calibrated. Also, the assessments are subjective measures of historical disease and are therefore difficult to use as a means of predicting the course and severity of progressive periodontal disease. Variables involved include probe dimensions, construction, placement and reference point, probing force, gingival tissue condition, tooth alignment and shape, and the presence of subgingival deposits.

In order to evaluate progressive inflammatory disease a series of clinical indices were developed during the 1960s and 1970s based upon evidence of tissue damage. Despite short comings, these indices remain in widespread use and provide a simple

means for determining and recording the progression of periodontal diseases over a period.

1.2.6.3.1 BPE - Basic Periodontal Examination

The Basic Periodontal Examination is a screening assessment to identify the presence or absence of periodontitis, it requires that the periodontal tissue should be examined with a standardised periodontal probe using light pressure to test for bleeding, plaque retention and pocket depth. The dental arches are divided into sextants and the worst score per sextant is recorded using a hierarchical index.

1.2.6.3.2 Plaque levels

The plaque index is a clinical measure of the quantity of plaque at a specific tooth site. Variations include the Plaque index (Loe, 1967) and the Turesky modification of the Quigley Hein index (Turesky et al., 1970). The latter is one of the most commonly used. Orthodontic tooth movement requires appliances which are bulky and plaque retentive and so Ciancio et al. (1985) developed a bonded bracket plaque index to take account of this.

1.2.6.3.3 Severity of gingivitis

The gingival index is a qualitative clinical measure of inflammatory changes within the gingival tissues (Loe, 1967).

1.2.6.4 Force delivery systems to generate orthodontic tooth movements

Three variables affect the biological response to orthodontic force: force magnitude; duration; and means of application.

1.2.6.4.1 Force magnitude

Light forces have been recommended for efficient and less painful tooth movement (Hixon et al., 1969). However the rate of tooth movement bears little relation to force magnitude (Andreason and Zwanziger, 1980). It can therefore be suggested that the importance of light forces lies not in the production of an optimal rate of tooth movement, but in limiting the undesirable side effects of heavy forces which increase the risk of radicular damage, however the forces that produce these side effects vary amongst individuals making it difficult to discern what a heavy force is (Owan-Moll et al., 1996). Optimal forces for given tooth movements are listed in Table 1.1. The surface area within the periodontium changes along the length of the tooth as teeth taper apically, therefore the forces required for intrusive movements are very low.

Table 1. 1 Ideal force levels to achieve different types of tooth movement (taken from Laura Mitchell. An Introduction to Orthodontics. 2nd Edition. Oxford. 2001)

Type of movement	Force required (g)
Tipping	30-60g
Bodily movement	100-150g
Rotation	50-75g
Extrusion	50-75g
Intrusion	15-25g

1.2.6.4.2 Force duration

A light, continuous force is the most efficient for producing tooth movement and heavy, continuous forces should be avoided. Interaction between force magnitude and the duration is complex since the applied force components can be subject to rapid force decay with time, especially those made from elastic polymers. Nickel-Titanium (NiTi) wires used in the initial phases of treatment do not obey Hooks Law i.e. the loading/unloading curve is not a straight line.

1.2.6.5 Mechanisms of force application

Nickel-Titanium alloys have two properties; super-elasticity and shape memory. These properties offer advantages during orthodontic tooth movements. Super-elasticity is the ability to demonstrate a non-linear loading curve such that the wire will exert the same force whether it is deflected a short or long distance. Constant force delivery over a range of activation during use is thought to be an efficient method of force delivery (Miura et al., 1988).

1.2.7 Force application measurement

The simplest method for measuring force is with the use of an intraoral Corex tension gauge. Several studies have compared different means of force application to find the most efficient means of space closure (Dixon et al., 2002; Nightingale and Jones, 2003). All of these studies have concluded that the NiTi coil springs produce the most constant and effective force and this is the method of force application to be used in the present study.

1.2.8 Tooth movement measurement

Assessment of the rate of orthodontic tooth movement involves measurement of distance and time. It is difficult to determine the distance over which each tooth moves during treatment because all teeth involved in the appliance move by varying degrees in varying directions. Anatomical structures have been used as reference points, for example palatal rugae, although the identification of suitable fixed structures during appliance therapy can be problematic (Almeida et al., 1995). Baldwin et al., (1999) used a reflex metrograph at six weekly intervals to calculate the three dimensional tooth movements produced by NiTi archwires.

Another method of assessing tooth movement is to measure the linear displacement of anatomical contact points using a dial calliper. During orthodontic alignment the teeth line up so that contact point displacement reduces with time. This can be used to indicate the rate of achievement of alignment and hence act as an indicator for rate of tooth movement. Such a method was first developed by Little, (1975) and used as an indicator of stability in lower incisor position following orthodontic treatment over time.

1.2.9 Gingival crevicular fluid

1.2.9.1 The gingival crevice

The gingival crevice is a relatively fragile tissue barrier, which separates the internal tissues of the body from a hostile external environment, the mouth. In health this barrier is known as the junctional epithelium. It is non-keratinised and permeable and so allows materials from the mouth to pass into the periodontium and vice versa.

1.2.9.2 The formation of gingival crevicular fluid (GCF)

The gingival crevice contains fluid that is derived from beneath the gingival margin. In health this gingival crevicular fluid (GCF) is a serum transudate and therefore contains all of the components of serum and some cellular elements, in particular polymorphonuclear leucocytes (PMNL).

A local osmotic gradient is responsible for the movement of fluid from intravascular to perivascular sites. During the process various substances may be incorporated into the fluid, reflecting the biology and physiology of the surrounding tissues. In health GCF composition reflects the physiological processes underlying normal tissue turnover, in gingivitis and periodontitis it becomes an inflammatory exudate developed in response to inflammatory and immunological reactions. Similarly, during orthodontic tooth movement the composition of the fluid may alter to reflect periodontal connective tissue turnover.

1.2.9.3 The composition of gingival crevicular fluid

As a result of the permeability of the junctional epithelium, even in clinical health there exists a degree of infiltration of inflammatory cells in the periodontium. Once disease is established the gingival connective tissue becomes a site of acute inflammation. The cardinal signs of acute inflammation are seen clinically within the gingival tissue, which becomes red, swollen and oedematous, a cellular PMNL infiltration becomes evident. With time the normally thin junctional epithelium becomes punctuated with microulcerations and may bleed easily with applied pressure. Therefore, in gingivitis and other inflammatory diseases of the periodontium the serum transudate becomes an inflammatory exudate. As previously mentioned, during orthodontic treatment inflammatory reactions are also generated, one initiated by pressure gradients within the periodontal membrane, the second resulting from the microbial plaque challenge.

Substances that may be involved in connective tissue remodelling have been studied by measuring their concentrations in GCF. Last et al., (1988) found an increase in chondroitin sulphate (a type of glycosaminoglycan) on the side to which teeth were moved orthodontically. Grieve et al., (1994) found that levels of prostaglandin E and interleukin 1 β increased over a period of 24-48 hrs following the initiation of tooth movement. Lactic and citric acid levels also increase during tooth movement (Miyajima et al., 1991). Plasminogen activator and plasminogen activator inhibitor have been studied in health, gingivitis and periodontal disease (Xiao et al., 2000). These and other studies may enable measurements of the constituents of GCF to be used to monitor biological events in particular during orthodontic tooth movement.

1.2.9.4 Collection of gingival crevicular fluid

The most commonly used sampling method uses a paper strip inserted into the gingival crevice until mild tissue resistance is felt. It is left in position for a known period of time whilst GCF is absorbed into it (Brill and Krass, 1958). A sampling period of 30s has been adopted by most investigators with the use of a methyl cellulose collecting strip. The composition of GCF varies with the amount of gingival inflammation (Curtis et al., 1988). In order to illustrate the levels and change in levels of collected GCF compounds the sample may be assessed using the total amount collected over a pre-determined period. (Chapple et al., 1999)

1.2.9.5 Previous orthodontic studies using GCF

The presence/expression of regulatory proteins within GCF has been recognised as a measure for monitoring orthodontic treatment outcome. Reports have focused on the presence of new mediators and on regulation of the levels of these proteins.

Some studies have investigated one or two proteins only due to the limitations of the available assays. Different time points have been selected for sampling and different sampling methods used. Orthodontic appliances and movements vary between studies and different test teeth have been used. This makes it difficult if not impossible to compare studies many of which are under powered.

A meta-analysis on the role of GCF cytokines in orthodontic tooth movement has been attempted with a view to generating a time-related pattern for the production of GCF cytokines that may provide evidence at the cellular level. The meta-analysis was not justified due to the heterogeneity of the reports Many studies did not

discriminate between sites of tension and compression or only took samples from compression sites. (Ren and Vissink, 2008)

1.2.10 Inflammatory cytokines

Orthodontic tooth movement is a type of tissue injury leading to an inflammatory response (Davidovitch, 1991). Endothelial cells are activated and cytokines and chemoattractants that result in leukocyte activation are produced (Cooper and Sims, 1989). This interaction between endothelial cells and leukocytes is important in the inflammatory process, as it produces pro-inflammatory cytokines (PIC) and anti-inflammatory cytokines (AIC) (Maroszynska and Fiedor, 2000). PICs and AICs are bioactive molecules which maintain and regulate the inflammatory process by paracrine and autocrine signalling (Callard et al 1999). Cytokines that affect bone metabolism, and thereby orthodontic tooth movement, include interleukin1(IL-1), interleukin2(IL-2), interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor alpha (TNFα), interferon-γ (IFNγ), and osteoclast differentiation factor (ODF) (Krishnan and Davidovitch, 2006). Studies in bone remodeling have indicated that certain PICs, such as interleukins (IL-1b, -6, and -8) and tumor necrosis factor-alpha (TNF-a), are important regulators in the bone remodeling process upon mechanical stimulation (Ren et al., 2007). The most potent among these is IL-1, which directly stimulates osteoclast function through the IL-1 type 1 receptor, expressed by osteoclasts. IL-1 secretion is triggered by various stimuli, including neurotransmitters, bacterial products, other cytokines, and mechanical forces (Davidovitch, 1995). IL-1 has two forms coding different genes. IL-1 attracts leukocytes and stimulates fibroblasts, endothelial cells, osteoclasts, and osteoblasts to promote bone resorption and inhibit bone formation (Sabatini et al., 1988). Osteoblasts are target cells for IL-1, which in turn activate osteoclasts to resorb bone

(Davidovitch, 1995). IL-1β is a key mediator in a variety of activities in immune and acute-phase inflammatory responses (Dinarello, 1989).

IL-6 regulates the immune response at inflammation sites and stimulates osteoclast formation and the bone-bioabsorbing activity of preformed osteoclasts. (Okada and Murakami 1998; Dinarello, 1989**).** IL-8 regulates the influx of leukocytes at a site of inflammation. It also is a potent neutrophil chemoattractant and activator that is secreted by monocytes, macrophages, epithelial cells, and fibroblasts, all of which are abundant in the periodontal environment. (Ren et al., 2007). Tuncer et al., 2005 reported increased levels of IL-8 at PDL tension sites and proposed it to be a triggering factor for bone remodeling. TNFα, another pro-inflammatory cytokine, was shown to elicit inflammation and stimulate bone resorption. TNFα is synthesised and released by monocytes and macrophages by activation of leukocytes in vitro and is a potent inducer of bone resorption (Bertolini et al., 1986). Davidovitch et al., 1988; Satio et al., 1990; Alhashimi, 2000 and Alhashimi, 2001 have shown that TNFα directly stimulates the differentiation of osteoclast progenitors to osteoclasts in the presence of macrophage colony-stimulating factor (M-CSF). Davidovitch et al., (1988) and Saito et al., (1990) demonstrated marked increases in the staining intensity for IL-1 and TNFα in cells of the PDL and alveolar bone during orthodontic tooth movement.

In contrast to interferon-α and interferon-β, which can be expressed by all cells, interferon-γ (IFNγ) is secreted by T lymphocytes and natural killer cells only and is the only Type II interferon. It is serologically distinct from Type I interferons and it is acid-labile, while the Type I variants are acid-stable. IFNγ has antiviral,

immunoregulatory, and anti-tumour properties (Schroder et al., 2004). It alters transcription of at least 30 genes, producing a variety of physiological and cellular responses. Activation by IFNγ is achieved by its interaction with heterodimeric interferon γ receptors. IFNγ suppresses osteoclast formation by rapidly degrading the RANK adaptor protein TRAF6 in the RANK-RANKL signalling pathway, which otherwise stimulates the activation of the transcription factor NFκB.

1.2.11 Tissue metabolites

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases of the matrixin family (Nagase, 1999). Most are secreted as inactive enzymes (pro-enzymes) which are activated in the extracellular compartment or in the vicinity of other MMPs or serine proteases (Nagase, 1997). The proteolytic activity of MMPs is under the control of circulating and endogenous tissue specific inhibitors which control extent of extracellular matrix remodelling (Starkey and Barrett, 1973).

The main substrates of the gelatinases are Type IV collagen and gelatin, The gelatinases are MMP-2 and MMP-9. MMP-2, MMP-9 and TIMPs-1 and 2 are thought to be important in bone metabolism (Birkedal-Hansen et al., 1993).

1.2.12 Bone metabolites

RANKL (Receptor Activator for Nuclear Factor κ B Ligand) and OPG (osteoprotegenrin) are members of the TNF super family and regulate bone resorption (Lerner 2004). RANKL is membrane bound and can be found on osteoblasts, fibroblasts and activated T and B cells. Its osteoclastogenic action is blocked by the soluble decoy receptor OPG, whose expression is tightly regulated by systemic and local stimuli. RANKL is a principle osteoclast activator, produced by osteoblasts. Nulizygous mutant mice with deletion of the RANKL gene have been found to be osteopetrotic as there is no osteoclast activity due to the absence of an osteoblast signal. When RANKL protein was injected into these mice the ostopetrosis reversed. RANKL has also been shown to be important for the differentiation of osteoclasts from their monocytic precursors. RANKL increases and OPG levels decrease in GCF samples during orthodontic tooth movement (Mogi et al., 2004).

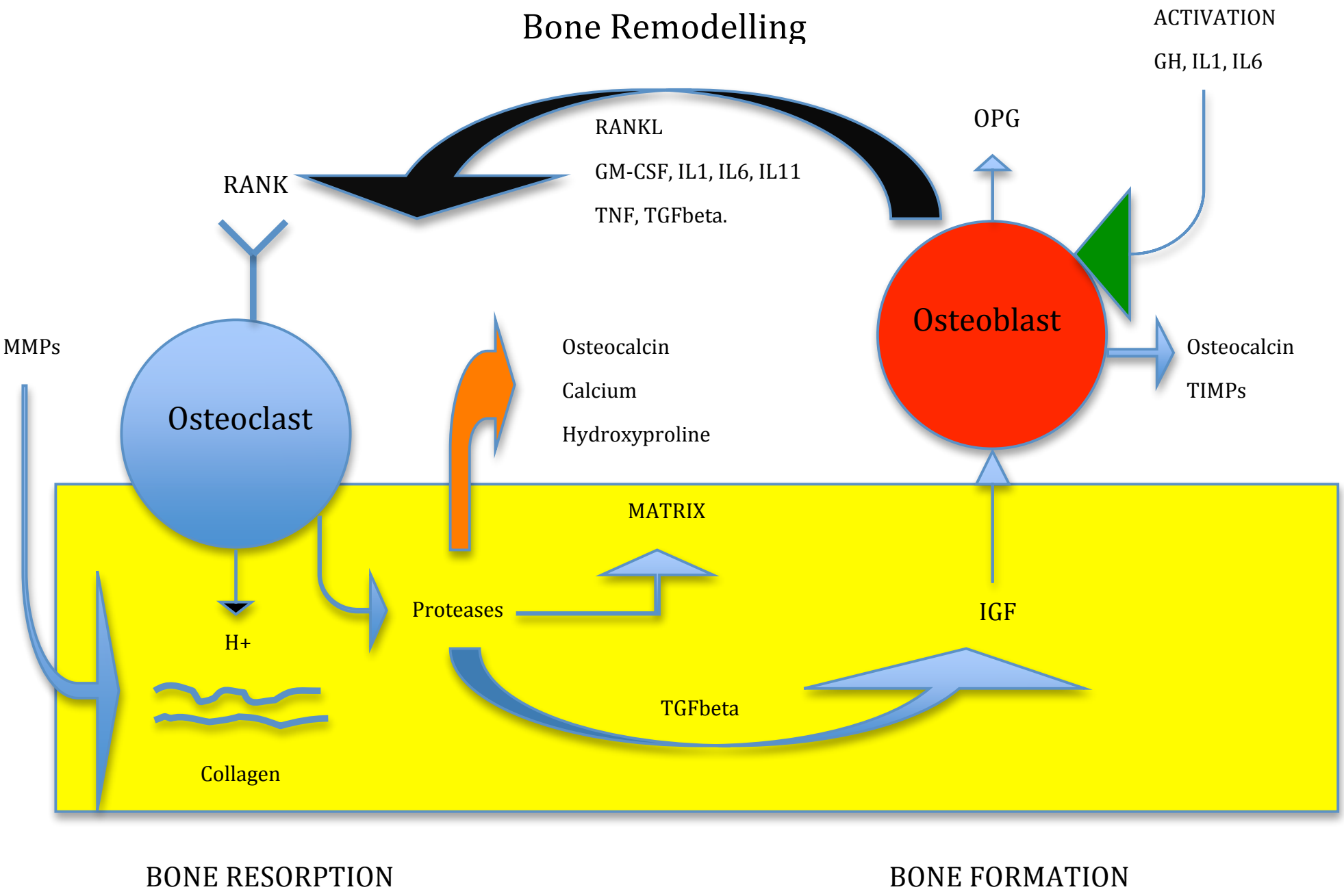


Figure 1.1 The bone remodelling cycle and metabolite interactions (adapted from a diagram at www.endotext.org)

1.3 Aims of the study

The aim of the present study is to investigate the effects of orthodontic tooth movement on the periodontium by analysing cytokine, protease and protease-inhibitor protein levels within GCF. Arrays will be employed to profile several biomarkers simultaneously within GCF and to correlate changes in protein profiles with differing applied forces and also between zones of tension and compression.

The objective of the proposed study is to improve our understanding of tissue changes induced by orthodontic forces, with view to a better understanding of differing rates of tooth movement and adverse events.

1.4 Hypotheses

The null hypotheses were that when an orthodontic force was applied to a tooth;

1) There is no change in the biomarker profile in GCF.

2) There is no difference in the GCF biomarker profiles at the compression and tension sites.

3) There are no correlations between the various cytokines tested and the rate of tooth movement.

Chapter 2

Materials & Methods

2.1 Materials

Orthodontic materials:

MBT prescription brackets (3M Unitek)

0.014 Nikel Titanium Archwires

0.018 Nikel Titanium Archwires

0.018 Stainless Steel Archwires

9mm NiTi closing coil springs

Intra oral tension guage (Correx)

Long ligatures

Elastomeric modules (TP orthodontics)

Alginate impression material

Impression trays

Periodontal materials:

WHO probe

Periopaper Strips ™

Periotron 8000 ™

Laboratory materials:

MMP-9 and TIMPs (1, 2, 3 & 4) were measured using Fluorokine MAP kits from R&D systems (Abingdon, UK).

RANKL and OPG were measured using LINCOplex kits from Millipore (Watford, UK).

Cytokines (GM-CSF, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10 and TNFα) were measured using the Cytokine 10-plex kit from Invitrogen (Paisley, UK).

2.2 Methods

2.2.1. Ethical approval

Prior to the commencement of the study ethical approval was obtained from the Coventry Research Ethics Committee (Reference number 08/H1210/72). Potential subjects and parents were given all the relevant information both verbally and in writing at their first attendance to the orthodontic clinic. They were then given at least a week to review and digest the information before being consented to join the study. Information and consent forms are shown in Appendix A1.

2.2.2 Patient recruitment

This is a pilot study. Due to the size and nature of this study and lack of previous data we could not calculate a sample size. However, studies of individual biomarkers demonstrate significant changes detected with time during periodontal disease activity on 10-20 volunteers, where change is measured longitudinally within the same subjects at a power of >0.08 ($P<0.05$) (Brock et al., 2004, Wright et al.,2003). Thus we aimed to recruit a minimum of 20 subjects and planned to do a post hoc sample size calculation to aid future research.

21 consecutive subjects were invited to take part in the study as they attended the Birmingham Dental Hospital to begin a course of orthodontic treatment. Inclusion and exclusion criteria are shown in Table 2.1.

Table 2.1 Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Aged 12-20 years	Smokers
Good systemic health	Have a full mouth plaque and bleeding score of >20%
Competent	Probing depth values >4mm
About to undergo a course of orthodontic therapy with fixed appliances	Radiographic evidence of periodontal bone loss
Requires removal of both upper first premolar teeth	Courses of anti-inflammatory or antimicrobial treatments taken within 1 month prior to study
Has upper canine teeth fully erupted within the oral cavity	

2.2.3 Treatment

A detailed periodontal examination was carried out, to record plaque index, bleeding on probing and bleeding index. Gingival crevicular fluid (GCF) samples were taken from the test teeth (the maxillary canine teeth) and upper arch impressions were taken. During the next few weeks the maxillary first premolar teeth were extracted and one week was allowed for gingival healing before attachment of the straightwire appliance. All subjects had the same bracket system (MBT prescription), elastomeric modules (TP orthodontics) and archwire sequence. After three months into appliance treatment and after tooth extraction, having followed a normal archwire progression (0.014 Nikel Titanium→0.018 Nikel Titanium →0.018 Stainless Steel). Archwire changes were made at 6 weeks and 10 weeks, the 0.018 Stainless Steel archwire being placed at 10 weeks. 4 weeks later the distalising force was applied to the test tooth. This delay was to ensure that the teeth had aligned onto the archwire before the distalising force was applied to move the canine tooth so that the biomarker changes detected were due to mesiodistal and not buccopalatal tooth movement. 4 weeks after the stainless steel archwire was placed, another detailed periodontal examination, upper arch impression and GCF collection was undertaken, (Test point T1). After samples were taken at T1 a 9mm NiTi closing coil spring was placed between hooks on the brackets of the upper canine and the first molar with the aid of a ligature. The ligature was tightened to produce a force of 100g according to the intra-oral tension guage. At T2, T3 and T4 detailed periodontal examinations, upper arch impressions, GCF collections and force adjustments were carried out (Figure 2.2).

Figure 2.1 A straightwire appliance with upper and lower Stainless Steel archwires.

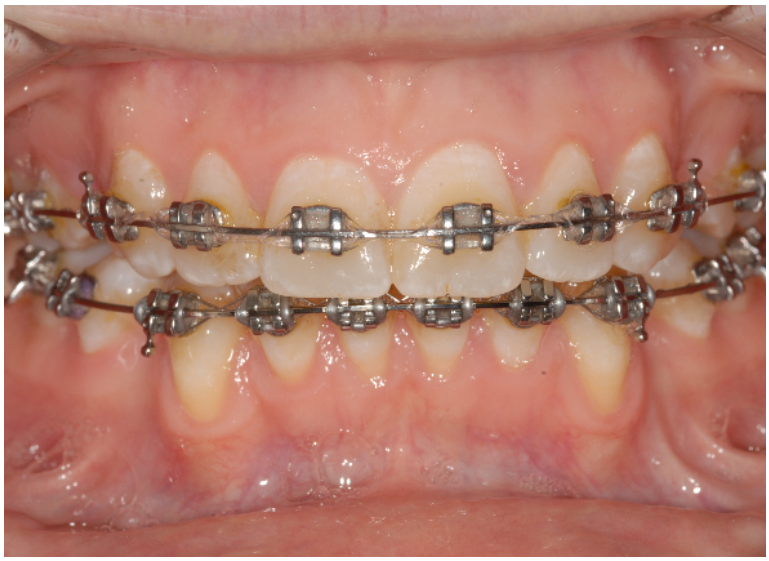
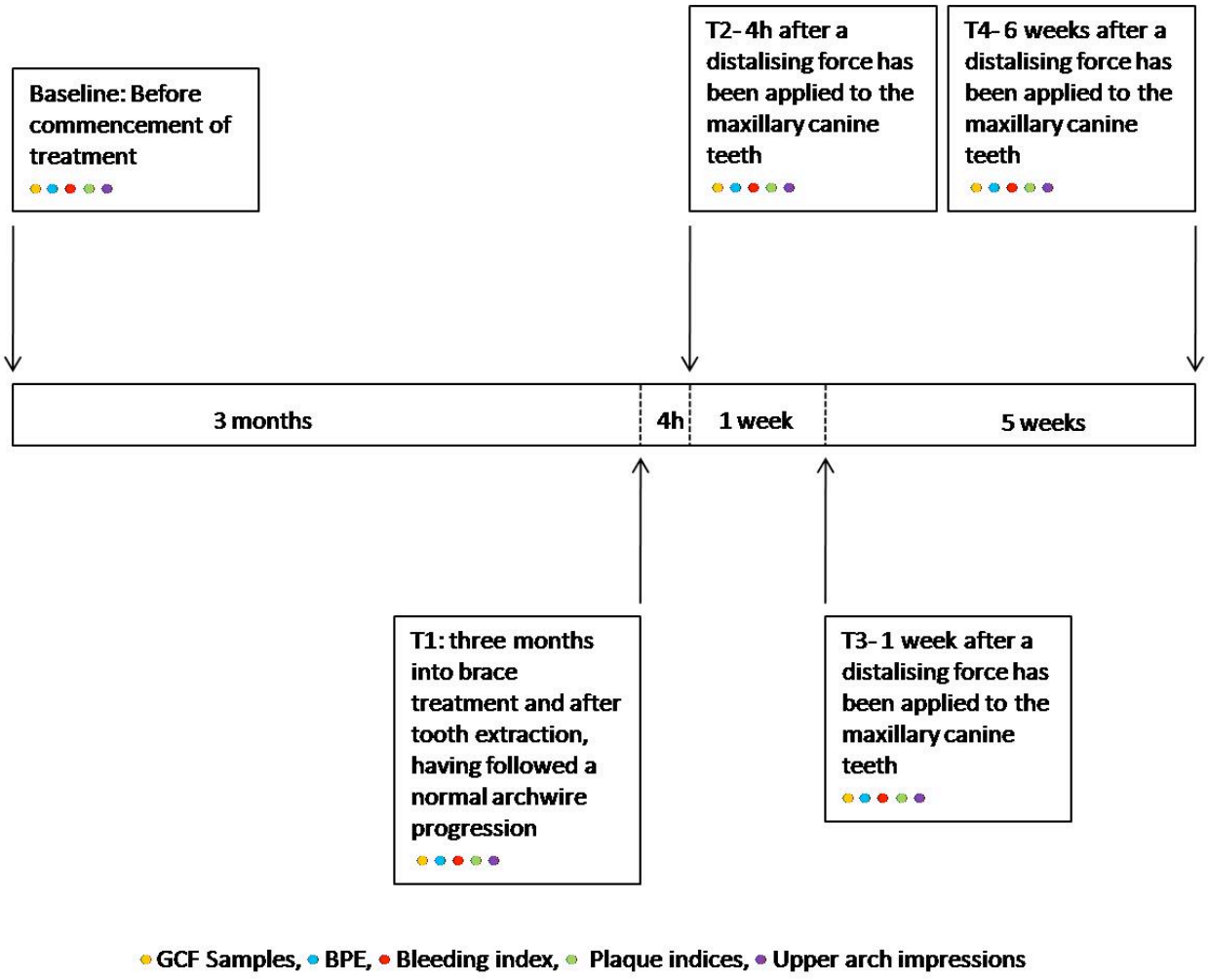


Figure 2.2 Summary of periodontal assessement and sample collection along the course of the study.



2.2.4 Gingival Crevicular fluid

2.2.4.1 Calibration of Periotron 8000™ .

The Periotron 8000 was calibrated by adding 35 known fluid volumes to periopaper strips, using Hamilton positive pressure syringes. The numerical readings given by the Periotron per volume were recorded. Each volume was tested thrice to minimise error and the Periotron was cleaned and dried between samples (Appendix B.1).

The mean value per volume was calculated and a calibration curve plotted. A fourth polynomial regression equation was formulated, from the mean values, and this formula was used to create a reference table that related volumes to the numerical readings shown on the Periotron 8000 (Chapple et al., 1999a) (Appendix B.2).

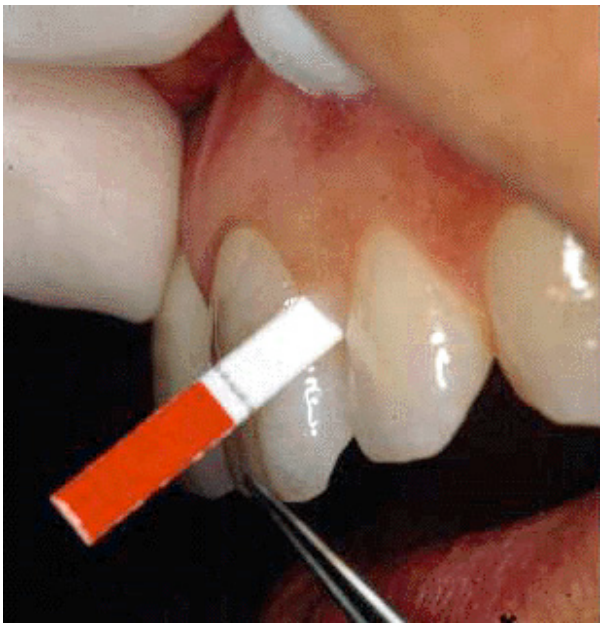
2.2.4.2 Collection of GCF

Prior to sample collection the test area was isolated with cotton-wool rolls and the teeth were air dried using a triple air syringe to remove saliva. The air was blown in a downwards direction to avoid drying the gingival crevice. GCF samples were collected using Periopaper strips ™ placed into the gingival crevice for 30 seconds until mild tissue resistance was felt (Brock et al., 2004). A Periopaper strip was then placed into the jaws of the calibrated Periotron 8000 to be read. Figure 2.3 shows the insertion of a Periostrip into a distobuccal position. In each subject and at each study time point individual Periopaper strips were placed into the gingival crevice at the mesiobucal and distopalatal aspects of both maxillary canine teeth. Samples were

then combined separately for compression and tension sites. Each pair of strips were eluted into sterile PBS (200 µl) and stored under liquid nitrogen until assay.

Mesiobuccal and distopalatal sampling sites were chosen to represent tension and compression. Because these are diametrically opposite, the risk of drawing GCF round the crevice from one site to the next is minimised.

Figure 2.3 Periostrip in a distobuccal position relative to the upper left central incisor.



2.2.5 Measurement of periodontal indicies

2.2.5.1 BPE (Basic Periodontal Examination)

Probing depth was measured as part of the BPE screen using a WHO (World Health Organisation) E-type probe. The gingival crevice surrounding each tooth was probed for bleeding, plaque retention and pocket depth. The following scores were applied:

0	No bleeding or pocketing or plaque retention detected
1	Bleeding on probing - no pocketing > 3.5mm and no plaque retention
2	Plaque retention present - no pocketing > 3.5mm
3	Pockets > 3.5mm but <5.5mm in depth
4	Pockets > 5.5 mm in depth

The dental arches were then split into sextants and the worse score per sextant was recorded as a general measure of periodontal health.

2.2.5.2 Bleeding index

Full mouth bleeding indices were performed at each visit as a measure of inflammation. The WHO E type probe was placed into the gingival margin of each tooth in turn and run around its circumference. Each tooth was then split into mesial, distal, buccal and palatal sites for recording purposes.

A score of 1 was given per site if bleeding was present and a score of 0 if it was absent. All scores were added to give the total number of sites expressed as a percentage.

$$\frac{\text{Number of sites scoring 1}}{\text{Total number of Sites}} \times 100 = \text{Percentage of the mouth with bleeding sites.}$$

2.2.5.3 Plaque levels

Full mouth plaque indices were obtained at each visit. A disclosing dye was applied to the teeth, the subject was then asked to rinse the mouth to remove excess dye.

A score of 1 was given per site if plaque was evident and 0 if it was not. Scores were added and the total number of sites calculated as a percentage.

$$\frac{\text{Number of sites scoring 1}}{\text{Total number of sites}} \times 100 = \text{Percentage of sites with plaque}$$

A separate more detailed plaque score was then calculated for each of the upper canines using the Tureskey modification of the Quigley Hein index:

Code 0= No plaque at the site examined following disclosure.

Code 1= A fine broken line of plaque present at the gingival margin of the examination site.

Code 2= A fine unbroken line of plaque present at the gingival margin of the examination site.

Code 3= Plaque covering up to a third of the crown from the gingival margin.

Code 4= Plaque covering up to two thirds of the crown from the gingival margin.

Code 5= Total tooth coverage.

This was recorded for the same four sites on these teeth (mesial, distal, buccal, palatal).

2.2.6 Impression taking

Alginate impressions of the upper arch were taken at all sampling stages in order to monitor tooth movement. The impression was then disinfected and moved into the laboratory for model casting.

2.2.7 Force application

Three months into the fixed orthodontic treatment, at T1, the 9mm NiTi coil springs were placed with the aid of a ligature, between the hook on the canine bracket and the hook on the first permanent molar attachment. The 9mm NiTi closing coils were then stretched, with the aid of the ligature and an intra oral tension guage (Correx) was used to ensure that 100g of force was being applied. At the following appointment the force was checked if necessary the ligature was tightened until the force returned to 100g.

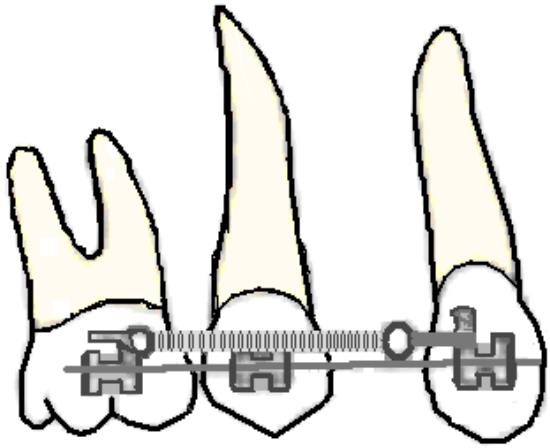


Figure 2.4 A 9mm NiTi closing coil spring stretched on a ligature between the upper canine and upper first molar (taken from http://www.orthocare.co.uk)

Figure 2.5 An intra oral tension guage (Correx) (taken from http://www.orthocare.co.uk)

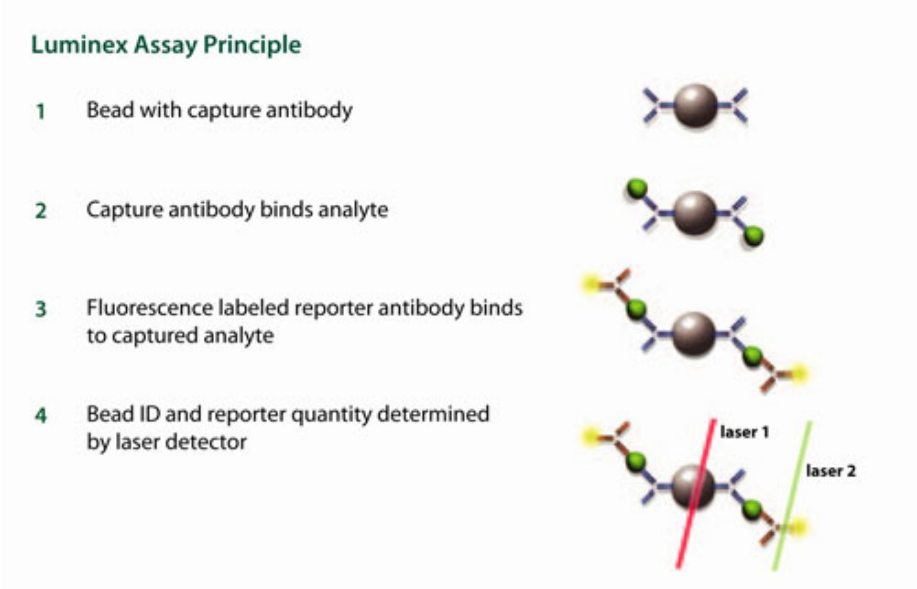
2.2.8 Multiplex assay

After collection, the GCF samples, were thawed on ice and aliquoted (50ul), so that repeated freeze-thaw cycles could be minimised. Biomarkers were detected using Luminex multi-analyte technology. Samples were defrosted and volumes of fluid extract were used for each assay (Table 2.2). Samples were incubated with antibodies immobilized on colour-coded microparticles, to detect molecules of interest, washed to remove unbound material and then incubated with biotinylated antibodies also to the molecules of interest. After further washing a streptavidin-phycoerythrin conjugate was added before a final washing to bind to the biotinylated antibodies. Figure 2.6 shows the capture of an analyte and the detection of that analyte by the recognition of the bead and the quantitation of the fluorescent phycoerythrin signal by two lasers. The Luminex analyzer determines the magnitude of phycoerythrin derived signal in a microparticle-specific manner.

Table 2.2 Volumes of GCF used in multiplex assays

Analytes	Volume of extract	Kit Manufacturer
TIMPs (1, 2, 3 & 4)	1µl	R&D
MMP-9	1µl	R&D
OPG	25µl	Millipore
RANKL	25µl	Millipore
Cytokines (GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN γ, TNFα)	50µl	Invitrogen

Figure 2.6 Principle of Luminex assay (taken from University of Pittsburg Cancer Institute website http://www.upci.upmc.edu/luminex/sources.cfm)



2.2.9 Statistics

Differences in individual GCF analytes across the course of the study were analyzed by non-parametric statistics (Kruskal-Wallis with Dunn's post test).

Chapter 3

Results

Clinical Indices

3.1 Introduction

When looking for changes in GCF it is important to record bleeding and plaque indices at all time points to know if the changes were due to alterations in GCF constituents following tooth movement and not due to confounding factors such as poor oral hygiene (indicated by high plaque and bleeding scores). Changes in GCF volumes were recorded in order to calculate biomarker levels as "total amount" per 30 second sample time as well as concentration.

This chapter presents clinical measurements taken at each time point to establish the status of the periodontal tissues during orthodontic treatment. Values include GCF volume, plaque index, gingival index and tooth movement.

3.2 Tooth movement

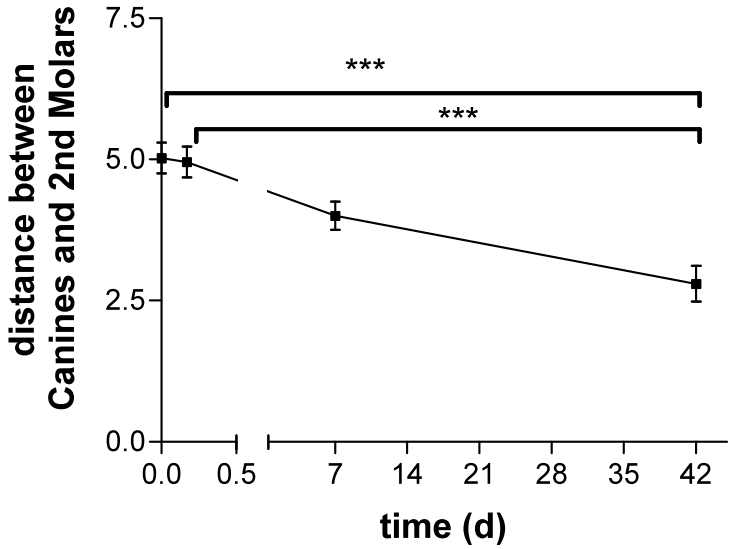
The distance between contact points on the upper canines and the second premolars was measured from study models. Measurements were repeated three months after the start. The percentage coefficient of variation for these measurements was low, showing high levels of intra-examiner agreement (CV % mean 0.17 range -6.45 to 8.54).

Averages of the two measurements were used for data analysis. The averages for left and right at each time point were combined and divided to give an average value of tooth movement of the upper canines (Figure 3.1A). There were significant changes ($p<0.001$) in tooth movement between T1 (before distalising force was applied) and

T4 (distalising force active for six weeks), and between time points T2 (distalising force active for four hours) and T4 (Figure 3.1A). A general trend can also be seen for space decreasing steadily from T2 to T3 to T4.

The speed of tooth movement was calculated by dividing the distance moved by the time taken (Figure 3.1B).

A



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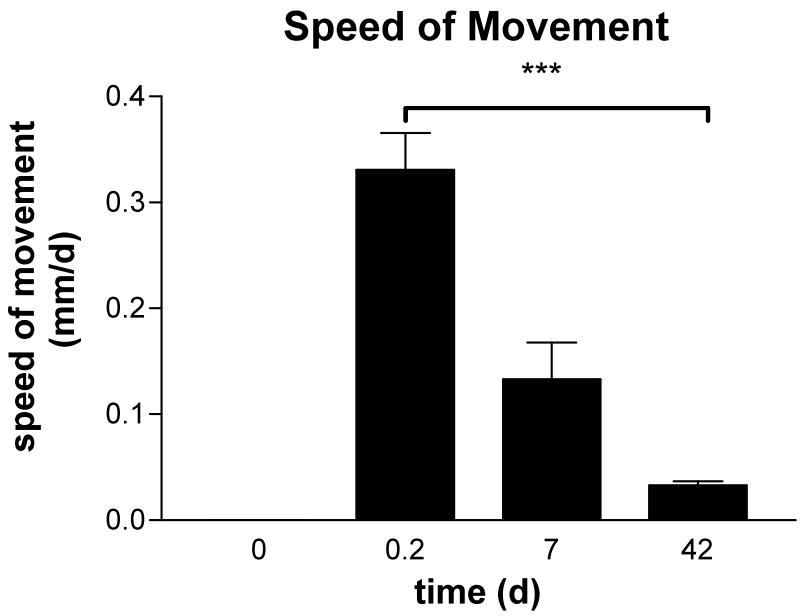


Figure 3.1A&B – Clinical measurements of tooth movement, from maxillary canines and the speed of tooth movement between the different time points(mean +/- sem). Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.

On the basis of the tooth movement results the subjects were divided into three even groups; those whose teeth moved fastest, those with medium movement and those with slow movement, so that other measurements might be stratified for speed of movement (Figure 3.2).

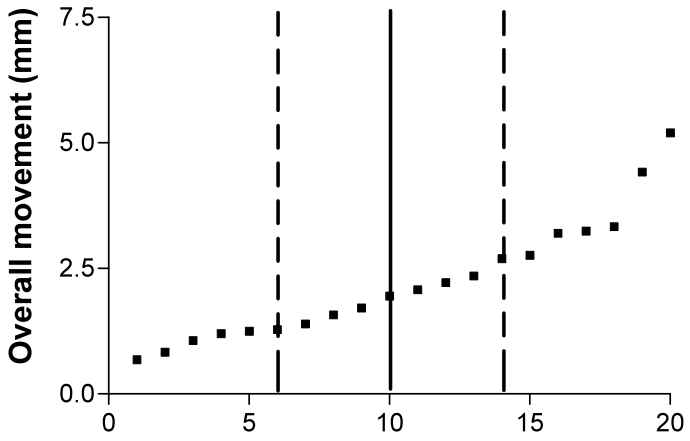


Figure 3.2 – Overall tooth movement, of maxillary canines (T4-T1) Dotted lines represent tertile divisions into the three tooth movement speed groups (slow, medium and fast movers) and parallel solid line represents the median.

3.3 Plaque index

There were no significant differences in full mouth plaque scores between any of the time points (Figure 3.3), demonstrating that appliance wear did not compromise plaque removal. Plaque levels around the upper canines and second molars did not alter with appliance placement or when a spring and distalising force was applied (Figure 3.4A). However, there was a trend indicating that the mesiobuccal aspect of the upper canines (tension site) harboured more plaque than the distopalatal aspect (compression site) (Figure 4A). The reverse can be said of the second molars with the mesiobuccal (tension site) harbouring more plaque (figure 3.4B).

Plaque scores at tension sites were found to be significantly greater at canines when compared to second molars at T1 ($P<0.001$) , T2 ($P<0.05$), T3 ($P<0.001$) and T4 ($P<0.01$). At the compression sites there was significantly more plaque on the second molars than the canines at T1($P<0.05$).

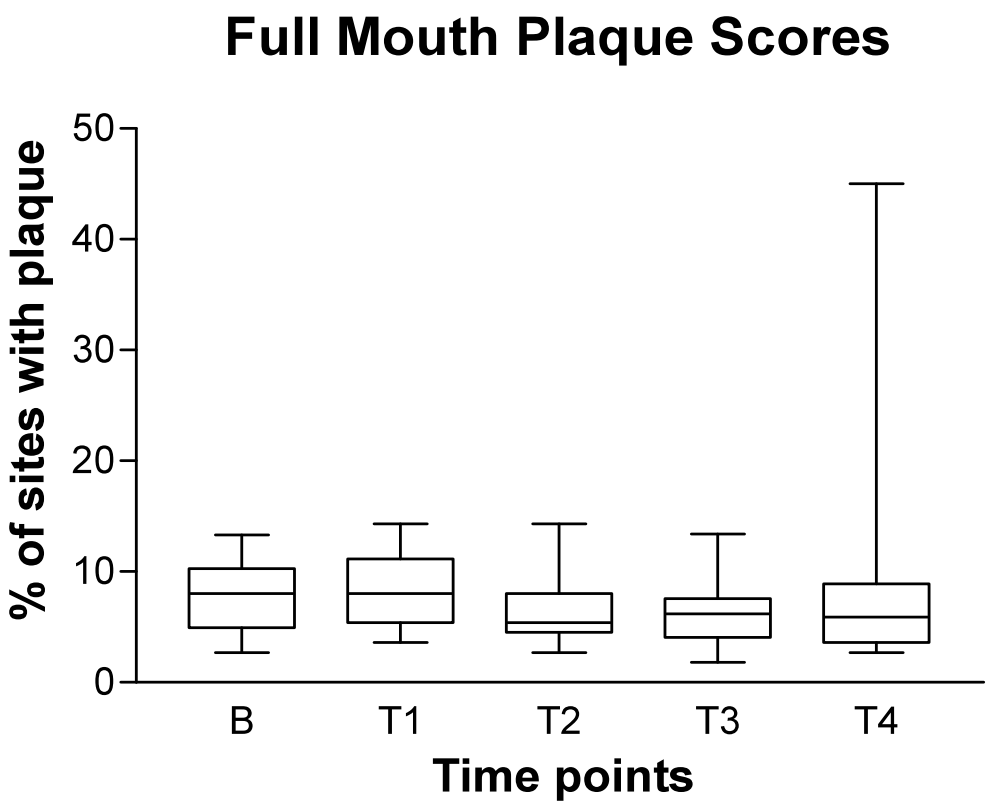
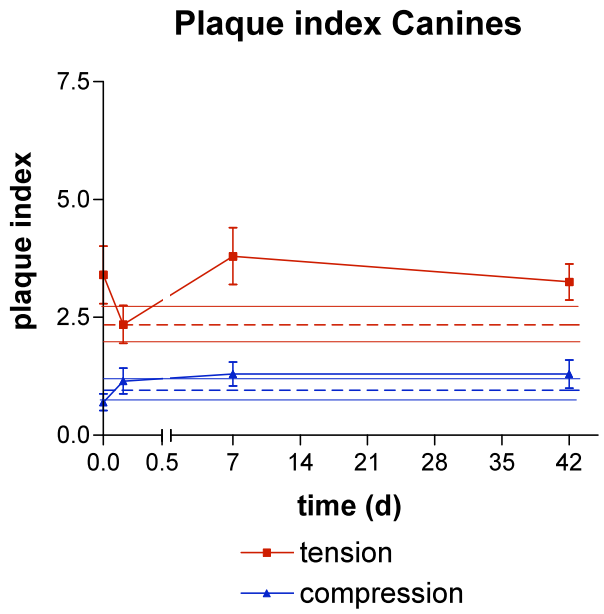


Figure 3.3 Full mouth plaque scores at each time point. Box and whisker plots represent median, 25% and 75% ranges and highest and lowest values.

A



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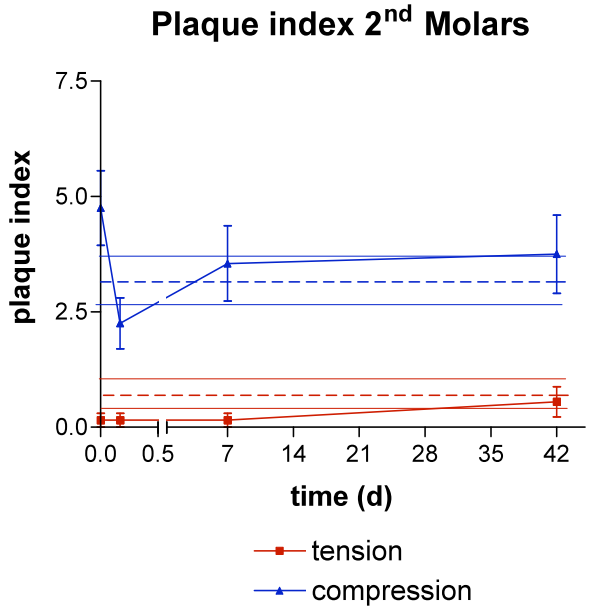


Figure 3.4A&B – Clinical values and scores for Plaque levels (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the sem of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

3.4 Gingival bleeding

Full mouth bleeding scores increased between baseline and T1, indicating that when appliances were placed bleeding scores increased (Figure 3.5). However, this trend was not found to be statistically significant. There was a significant decrease in bleeding between time points T1 and T2, when the distalising spring was placed

There was a significant ($p<0.05$) rise in bleeding mesiobuccal to canines between baseline and T1, when appliances were placed (Figure 3.6A). However the same change was not seen at the distopalatal site (Figure 3.6A). There were no significant changes between any of the other time points. At all time points the mesiobuccal aspect of the upper canines had a greater tendency to bleed than the distopalatal aspect (Figure 3.6A), perhaps indicating a difference in the tension and compression sites.

The second molars showed no significant changes between any of the time points or between tension and compression sites (Figure 3.7B). Bleeding scores at the tension sites were found to be significantly greater at canines than second molars at T1 ($P<0.001$) and T2 ($P<0.01$).

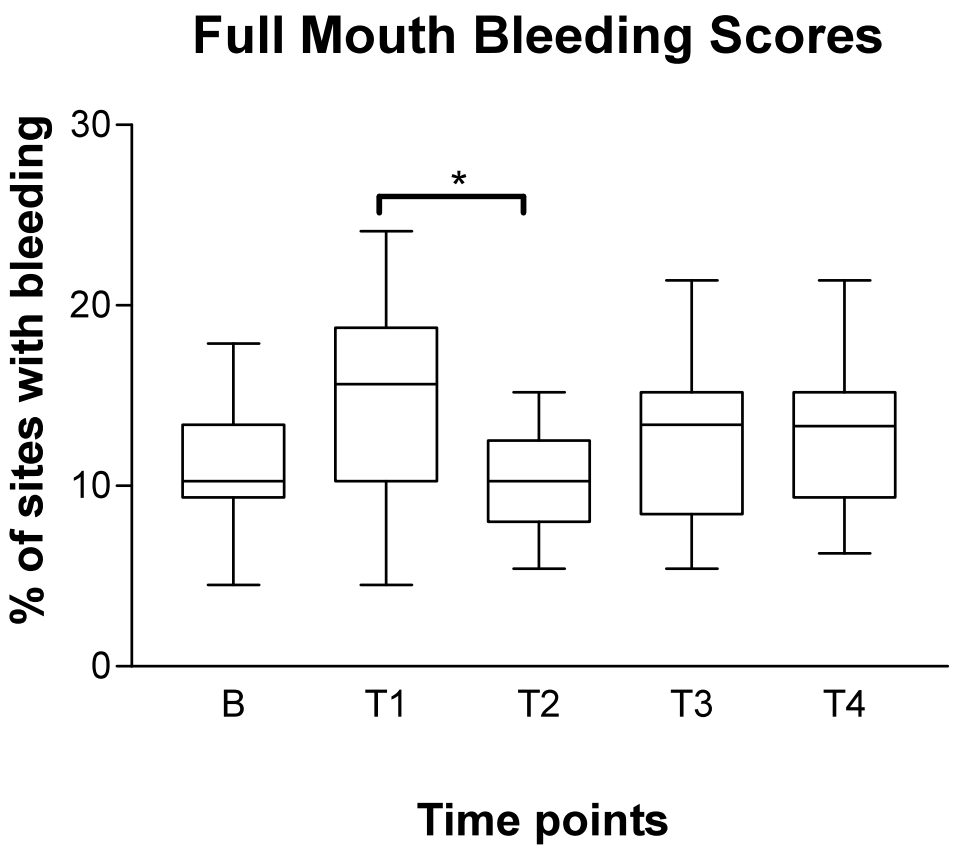
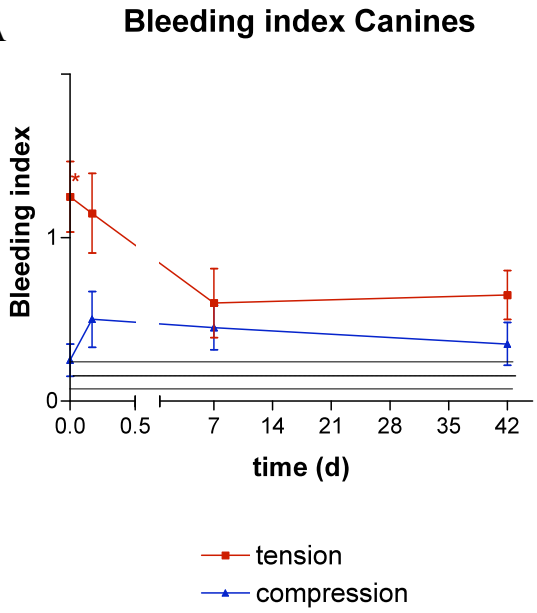


Figure 3.5 – Full mouth bleeding scores at each time point. Box and whisker plots represent median, 25% and 75% ranges and highest and lowest values.

A



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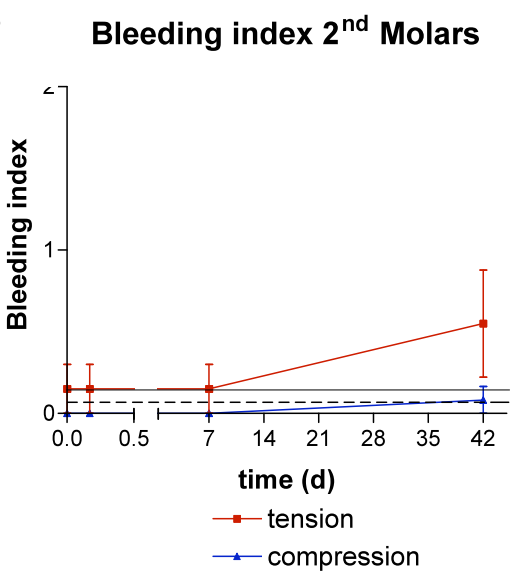


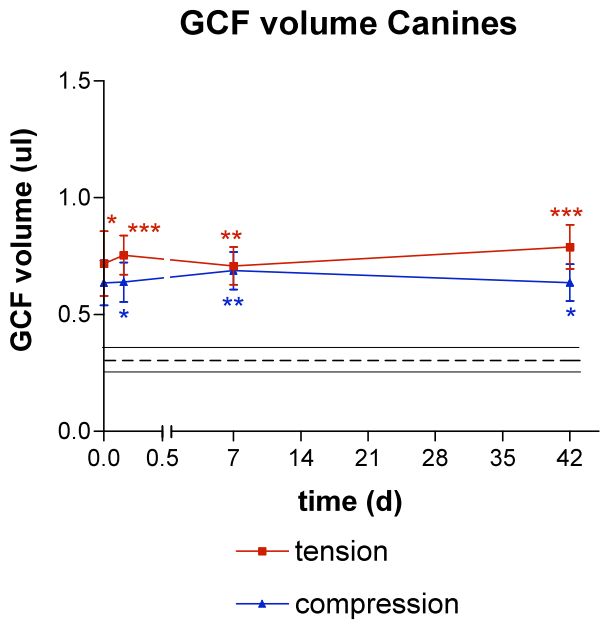
Figure 3.6A&B – Clinical values and scores for Bleeding levels, (mean +/-SEM) for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the sem of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

3.5 GCF volume

GCF volume increased at mesiobuccal canine sites between Baseline and T1 and between time points T1 and T2, when the spring was applied, before leveling out through time points T3 and T4 (Figure 3.7A). The changes between baseline and T1, T2, T3 and T4 were significant ($p<0.05$). No other volume changes between time points were significant. The GCF volume changes at the distopalatal aspects of the upper canines showed similar trends to the mesiobuccal sites, although the changes between Baseline and T1 were not significant (Figure 3.7A). However, changes between baseline and T2, T3 and T4 were significant ($p<0.05$). There was no statistical difference in GCF volumes between the compression (distopalatal) and tension (mesiobuccal) sites at any time (Figure 3.7A).

None of the volume changes at the second molar sites were significantat any of the time points. There was no significant difference between the sites of tension and compression around the second molars. GCF volume was not found to be significantly greater or less at canines when compared to second molars.

A



B

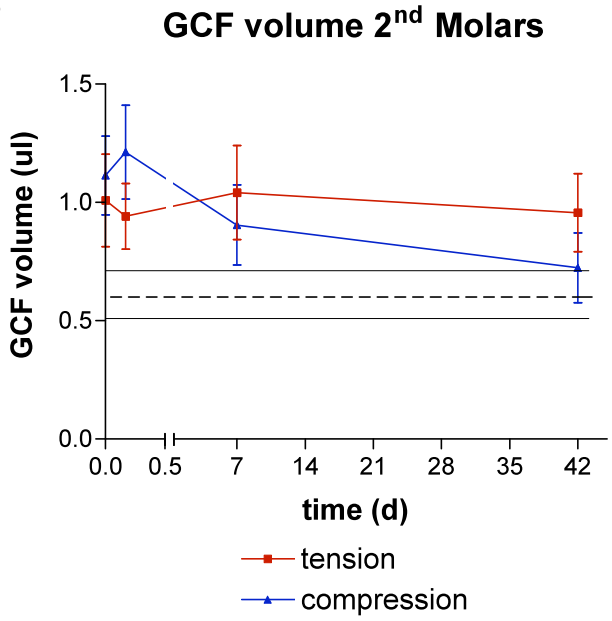


Figure 3.7A&B- Clinical values and scores for GCF volume, (mean +/-SEM) at sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the sem of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

3.6 GCF volume correlations

3.6.1 GCF correlations to plaque and bleeding scores.

There was a significant correlation between plaque scores and GCF levels at time points T1 for the canine tension site ($P<0.01$) and T1 for the canine compression site ($P<0.001$). None of the second molar plaque changes were significantly correlated with changes in the GCF volumes around those teeth. None of the correlation between bleeding scores and GCF volumes proved significant at both the canines and second molars (Table 3.1).

	Canine Tension Site		Canine Compression Site		second Molar Compression Site		second Molar Tension Site	
	Plaque Index	Bleeding Index	Plaque Index	Bleeding Index	Plaque Index	Bleeding Index	Plaque Index	Bleeding Index
Baseline			20	20	12	12	12	12
Number of XY Pairs	20	20	0.34	0.11	0.34	0.18	0.54	-0.31
Spearman r	0.33	0.22	0.14	0.65	0.28	0.57	0.08	0.33
P value (two-tailed)	0.15	0.36	Ns	Ns	ns	Ns	Ns	ns
T1								
Number of XY Pairs	20	20	20	20	12	12	12	12
Spearman r	**0.61**	0.15	**0.48**	0.23	-0.14	-0.25	-0.04	No value
P value (two-tailed)	**0.004**	0.52	**0.03**	0.33	0.67	0.43	0.90	
T2								
Number of XY Pairs	20	20	20	20	12	12	12	12
Spearman r	0.23	0.21	0.15	0.02	-0.51	0.25	-0.13	No value
P value (two-tailed)	0.33	0.38	0.52	0.94	0.09	0.42	0.68	
T3								
Number of XY Pairs	20	20	20	20	12	12	12	12
Spearman r	0.31	0.24	0.03	0.28	0.14	-0.46	0.31	No value
P value (two-tailed)	0.19	0.29	0.92	0.23	0.65	0.13	0.33	
T4								
Number of XY Pairs	20	20	20	20	12	12	12	12
Spearman r	0.002	0.13	0.14	0.24	-0.30	-0.21	0.09	-0.39
P value (two-tailed)	0.99	0.58	0.57	0.32	0.34	0.51	0.78	0.21

Table 3.1 – Comparisons of GCF volume changes to plaque and bleeding scores at tension and compression sites around the maxillary canines and second molars. Statistical analysis using Spearman Rank Correlation.

3.6.2 GCF correlations to speed of tooth movement.

No significant correlations were found between GCF volume and speed of tooth movement at any time within or between tension and compression sites around both canines and second molars.

Chapter 4

Results

Cytokines

4.1 Introduction

This chapter presents cytokine levels measured from GCF collected during the orthodontic treatment. Ten cytokines were assayed; GM-CSF, INFγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, and TNFα. Four were beneath the detection levels of the assays and six will be reported. Correlations between cytokine levels and clinical measures were analysed by Spearman Rank Correlations.

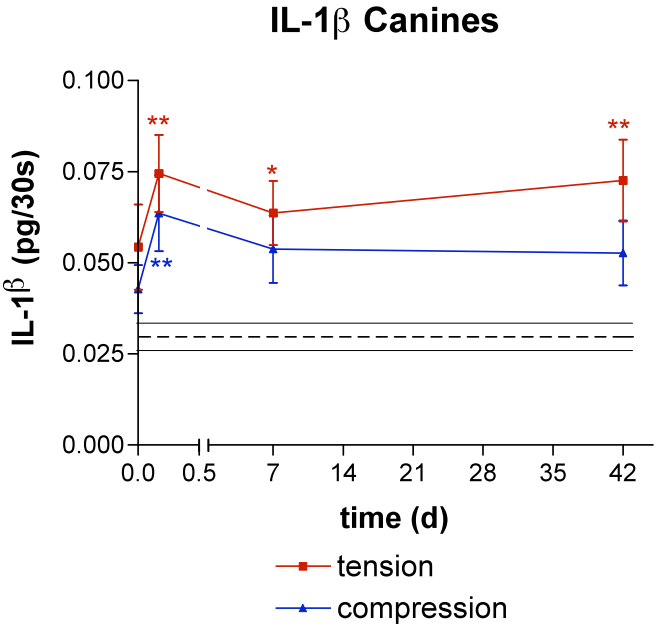
4.2 Interleukin-1β

There were significant differences between the levels of IL-1β expressed mesiobuccally to the upper canine, between Baseline and T2 ($p<0.01$), Baseline and T3 ($P<0.05$) and Baseline and T4 ($P<0.01$) (Figure 4.1A). Significant differences between the levels of IL-1β expressed distopalatally to the upper canine were found between Baseline and T2 only ($p<0.01$) (Figure 4.1A).

There were no significant differences between the levels of IL-1β expressed either mesiobucally or distopalatally to the upper second molars, between any time points.

There were no statistically significant differences in the amount of IL-1β between the mesiobuccal and distopalatal aspects of the canines or second molars at any of the time points. Figure 4.1A indicates a trend for lower levels of IL-1β at the distalpalatal (compression) sites at all time points except baseline, where they were very similar to mesiobuccal (tension) sites.

A



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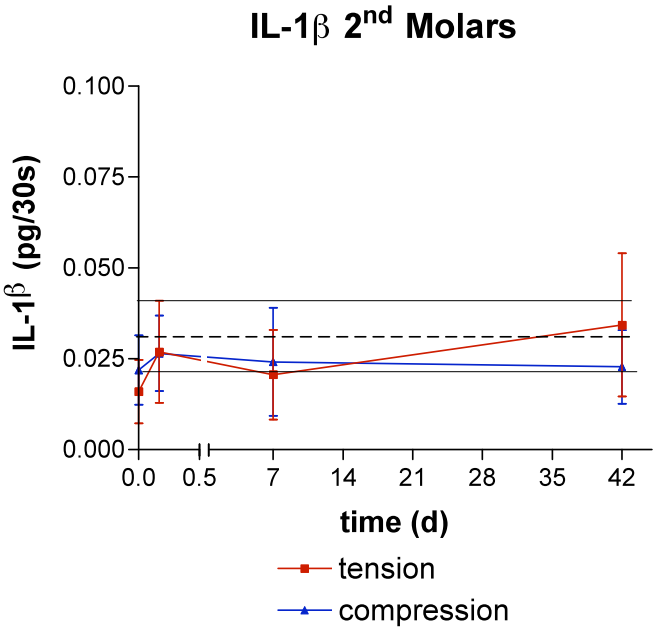


Figure 4.1A&B – IL-1β levels (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

4.2.1 Associations between clinical indices and IL-1β levels.

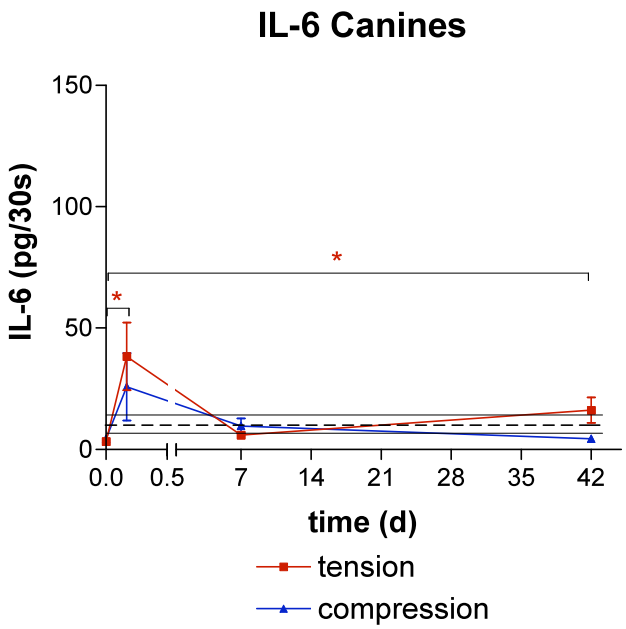
	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	ns	ns
Canine CS	*	*	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	B	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

Table 4.1 Correlations between IL-1β ,plaque and bleeding indicies and the rate of tooth movement. TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

4.3 Interleukin-6

There was a significant increase in the level of IL-6 between Baseline and T1 ($P<0.05$) and between Baseline and T4 ($P<0.05$) at the mesiobuccal canine site. There was no significant difference in IL-6 levels between any of the time points at the distopalatal canine site.(Figureure 4.2A). There were no significant differences in IL-6 levels between any of the time points at the mesiobuccal or distopalatal second molar site.(Figureure 4.2B). There were no statistically significant differences in the amounts of IL-6 between the mesiobuccal and distopalatal aspects of the canines or second molars at any time (Figure 4.2A&B).

A



B

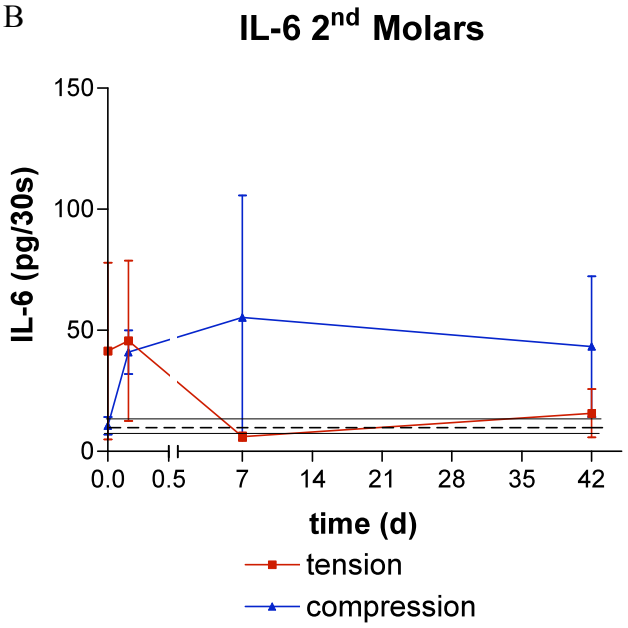


Figure 4.2A&B – IL-6 levels (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

4.3.1 Associations of clinical indicies with IL-6 levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

Table 4.2 Correlations between IL-6, plaque and bleeding indicies and the rate of tooth movement. TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

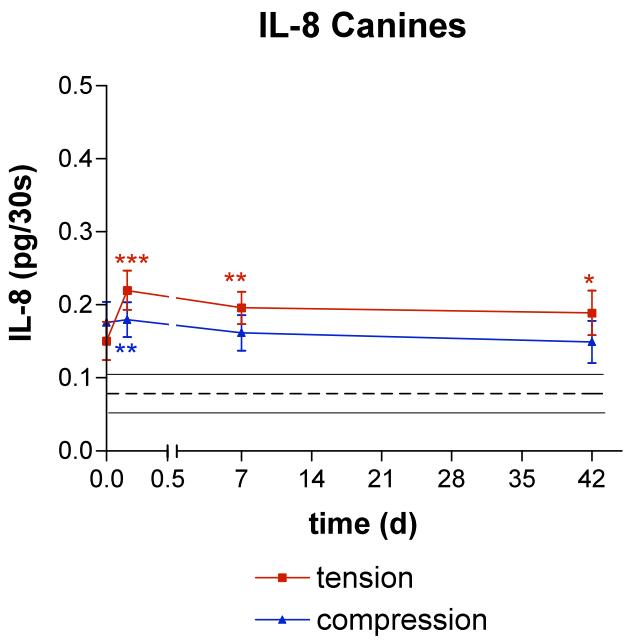
4.4 Interleukin-8

There was a significant increase in IL-8 levels between Baseline and T2 ($P<0.001$). Levels of IL-8 were also significantly higher than at Baseline at T3 ($P<0.01$) and T4 ($P<0.05$) (mesiobuccal tension canine sites). There was also a significant increase in IL-8 levels between Baseline and T1 ($P<0.05$) at the distopalatal canine site (Figure 4.3A).

There were however no statistically significant differences in the amount of IL-8 between the mesiobuccal and distopalatal aspects of the canines at any time (Figure 4.3A).

There were no significant differences in the amount of IL-8 between any of the time points at the mesiobuccal and distopalatal second molar sites. There was no statistically significant difference in the amount of IL-8 between the mesiobuccal and distopalatal aspects of the second molars at any time (Figure 4.3B).

A



B

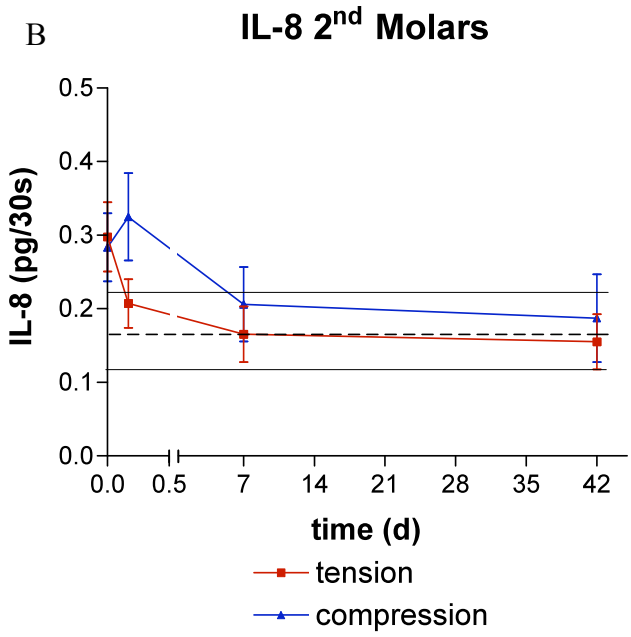


Figure 4.3A&B – IL-8 levels (+/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

4.4.1 Associations between clinical indices and IL-8 levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	*	ns	ns	ns
Canine CS	ns	*	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

Table 4.3 Correlations between IL-8, plaque and bleeding indicies and the rate of tooth movement. TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

4.5 Tissue Necrosis Factor α

Significant differences were found in TNFα levels at mesiobuccal sites between Baseline and T2 ($P<0.05$) and Baseline and T4 ($P<0.01$). No statistically significant differences were found in TNFα levels at any time for the distobuccal sites. (Figure 4.4A)

There were no statistically significant differences in the amount of TNFα between the mesiobuccal and distopalatal aspects of the canines at any time studied (Figure 4.4A).

There was a significant difference in the amount of TNFα between Baseline and T1 ($P<0.05$) and Baseline and T2 ($P<0.01$), at mesiobuccal second molar sites. No statistically significant differences were found at distobuccal second molar sites. There was no statistically significant difference in the amount of TNFα between the mesiobuccal and distopalatal aspects of the second molars (Figure 4.4B).

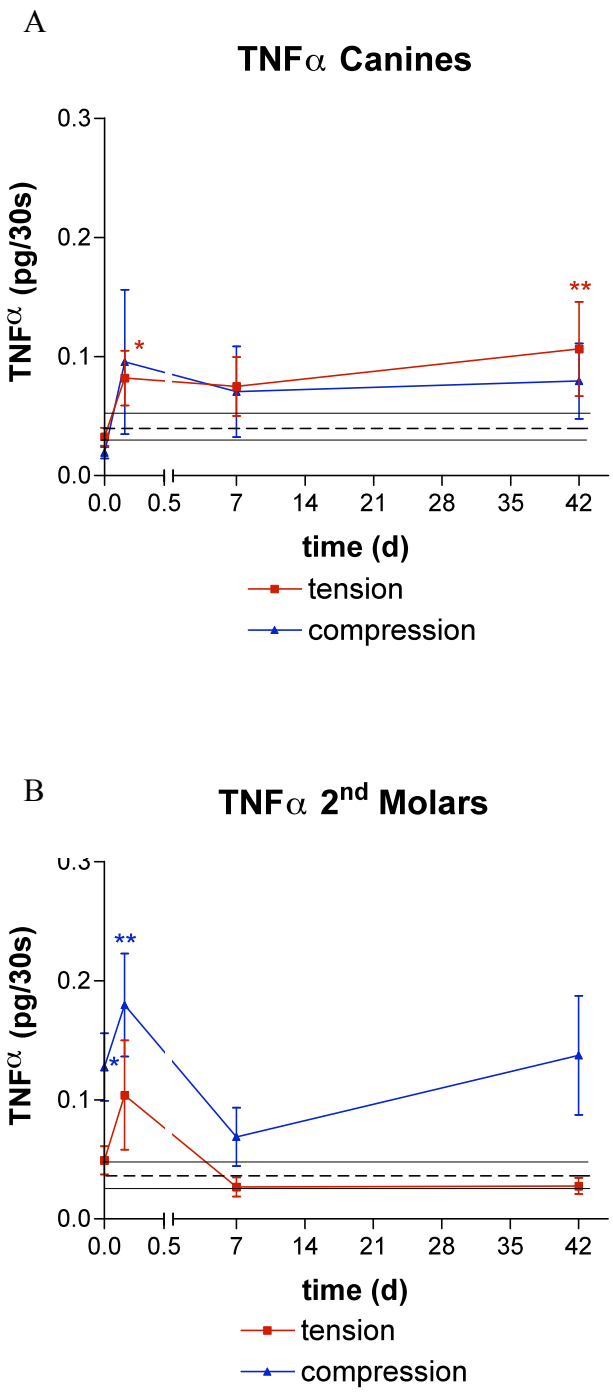


Figure 4.4A&B – TNFα levels (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

4.5.1 Associations between clinical indices and TNFα levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	*	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	*	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

Table 4.4 Correlations between TNFα, plaque and bleeding indicies and the rate of tooth movement. TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

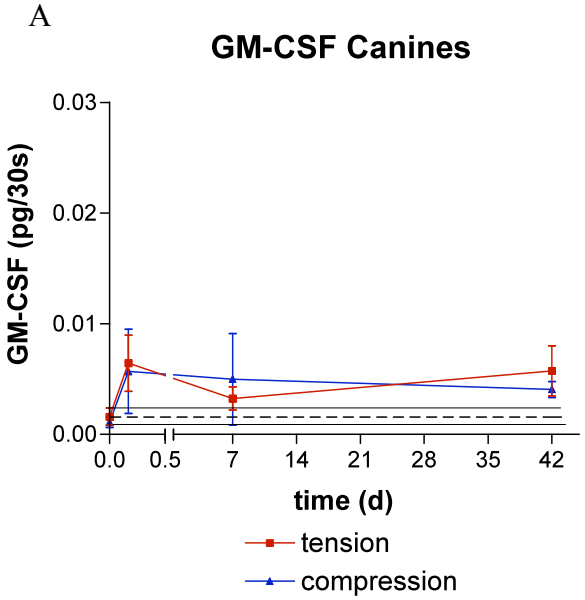
4.6 Granulocyte-Macrophage Colony-Stimulating Factor

No statistically significant differences were found in the levels of GM-CSF between any times at any sites.

There were no statistically significant differences in the amount of GM-CSF between the mesiobuccal and distopalatal aspects of the canines at any time (Figure 4.5A).

There was a significant difference in the amount of GM-CSF between Baseline and T1 ($P<0.05$) at mesiobuccal second molar sites. There were no significant differences in the amount of GM-CSF between any time points at the distopalatal second molar sites. There were no statistically significant differences in the amount of GM-CSF between the mesiobuccal and distopalatal aspects of the second molars at any time (Figure 4.5B).

A



B

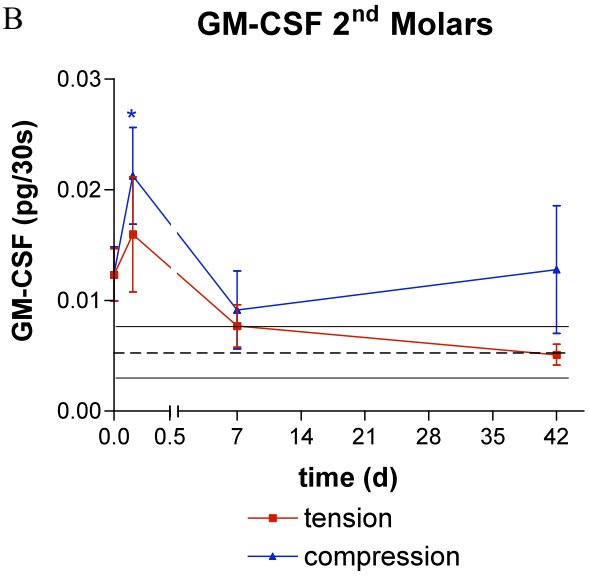


Figure 4.5A&B – GM-CSF levels (mean +/-SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

4.6.1 Associations between clinical indices and GM-CSF levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	*	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

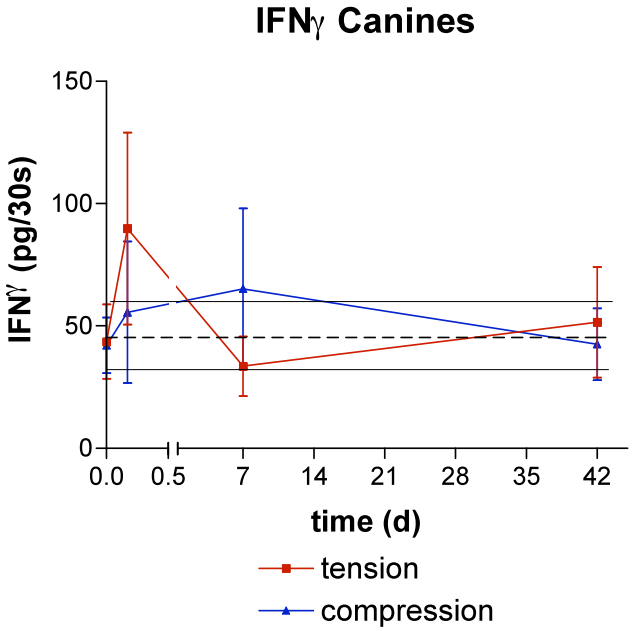
Table 4.5 Correlations between GM-CSF, plaque and bleeding indicies and the rate of tooth movement. TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

4.7 Interferon γ

No significant differences were found for the levels of IFNγ at either site at any time. There were no statistically significant differences in the amount of IFNγ between the mesiobuccal and distopalatal aspects of the canines at any time (Figure 4.6A).

There were no significant differences in the amount of IFNγ at any time at the mesiobuccal or distopalatal second molar sites. There were no statistically significant differences in the amount of IFNγ at the mesiobuccal and distopalatal aspects of the second molars at any time points (Figure 4.6B).

A



B

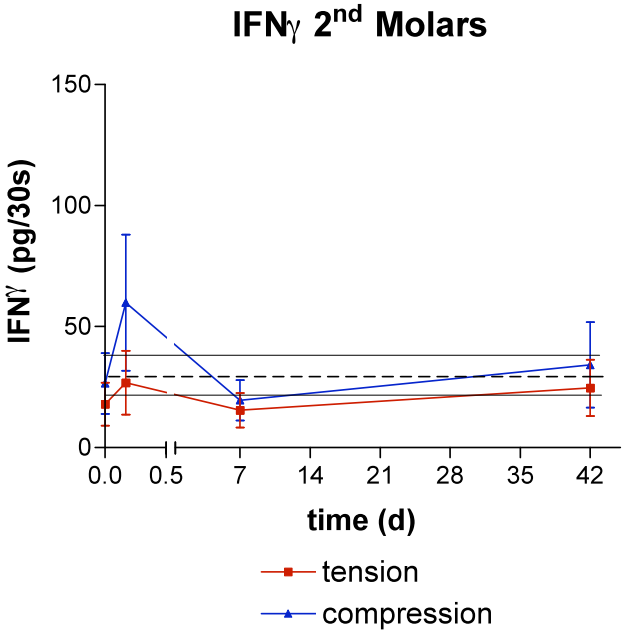


Figure 4.6A&B – IFNγ levels (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

4.7.1 Associations between clinical indices and IFNγ levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	*	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	*	*	ns	ns
Bleeding					
Canine TS	*	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

Table 4.6 Correlations between IFNγ, plaque and bleeding indicies and the rate of tooth movement. TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

Chapter 5

Results

Tissue Metabolites

5.1 Introduction

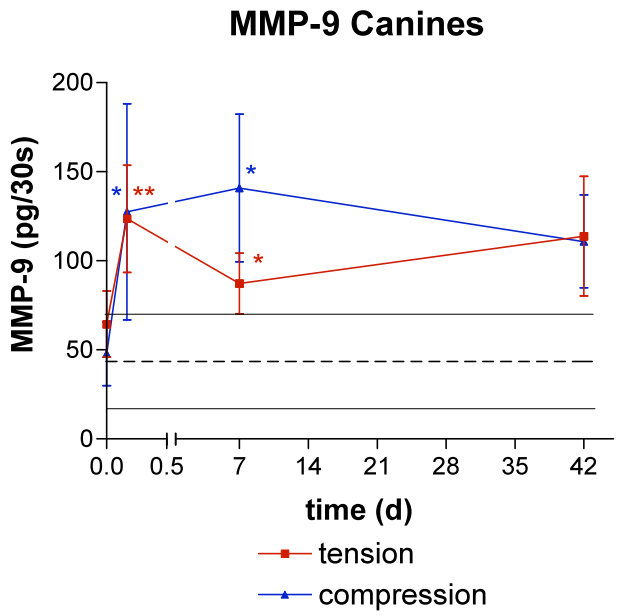
This chapter presents findings for tissue metabolites measured in GCF taken at all time points during the orthodontic treatment. Five tissue metabolites were assayed; MMP-9, TIMP-1, TIMP-2, TIMP-3, TIMP-4. However, TIMPs 3 and 4 were beneath the detection levels of the assays. The three detectable compounds and their ratios will now be reported upon. Correlations between metabolite levels and clinical measures were analysed using Spearmans Rank Correlations, unless otherwise stated.

5.2 Matrix Metallopeptidase-9

There were significant differences between the levels of MMP-9 expressed mesiobuccally to the upper canine, between time points; Baseline and T2 ($p<0.01$) and Baseline and T3 ($P<0.05$) (Figure 5.1A). There were also significant differences between the levels of MMP-9 expressed distopalatally to the upper canine, between Baseline and T3 ($p<0.05$) and Baseline and T4 ($p<0.05$) (Figure 5.1A). Significant differences were found in MMP-9 levels around the second molars at the mesiobucal (compression) site from Baseline at T1 ($P<0.05$) and T2 ($P<0.01$) (Table 5.1B).

There were no statistically significant differences in the amount of MMP-9 from the mesiobuccal and distopalatal aspects of the canines or second Molars at any time.

A



B

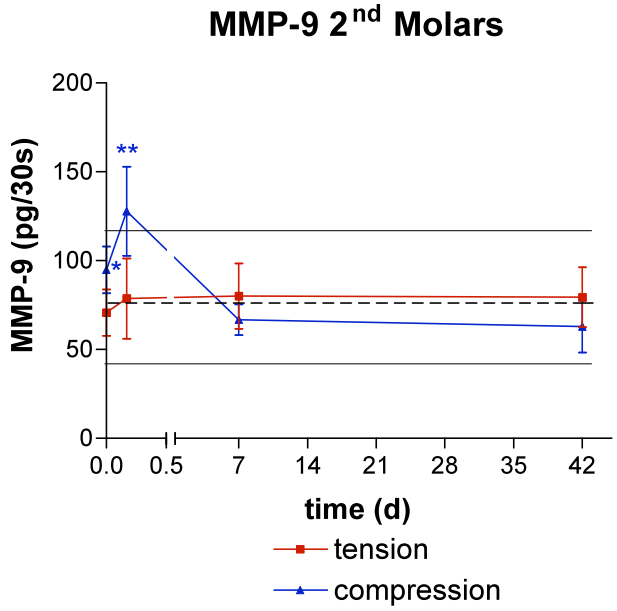


Figure 5.1A&B – MMP-9 levels (+/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

5.2.1 Associations between clinical indices and MMP-9 levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	ns	ns
Canine CS	*	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

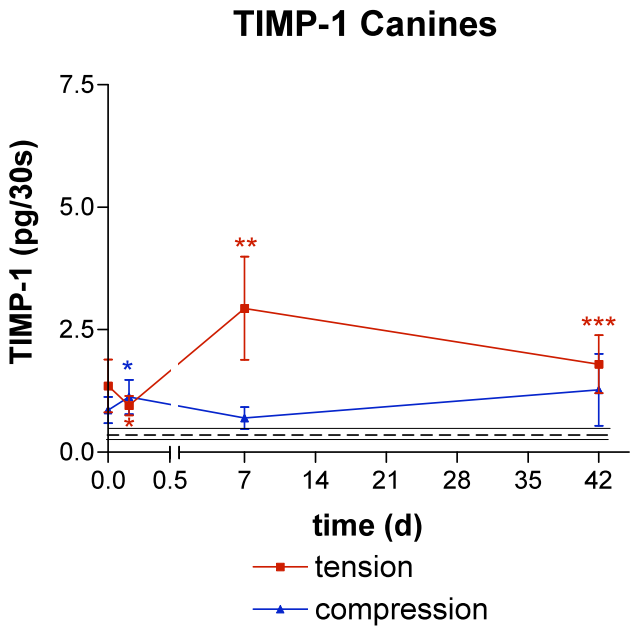
Table 5.1 Correlations between MMP-9, plaque and bleeding indicies and the rate of tooth movement. . TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

5.3 Tissue Inhibitor of Metalloproteinases-1

There were significant differences between the levels of TIMP-1 expressed mesiobuccally to the upper canine, between time points Baseline and T2 ($p<0.05$), Baseline and T3 ($P<0.01$) and Baseline and T4 ($P<0.001$) (Figure 5.2A). There were also significant differences between the levels of TIMP-1 expressed distopalatally to the upper canine, between Baseline and T2 ($p<0.05$) (Figure 5.2A). There was a significant difference between TIMP-1 levels at Baseline and T3 at the second molar mesiobuccal (compression site) ($P<0.05$) (Figure 5.2B).

There were no statistically significant differences in the amount of TIMP-1 between the mesiobuccal and distopalatal aspects of the canines or second molars at any time.

A



B

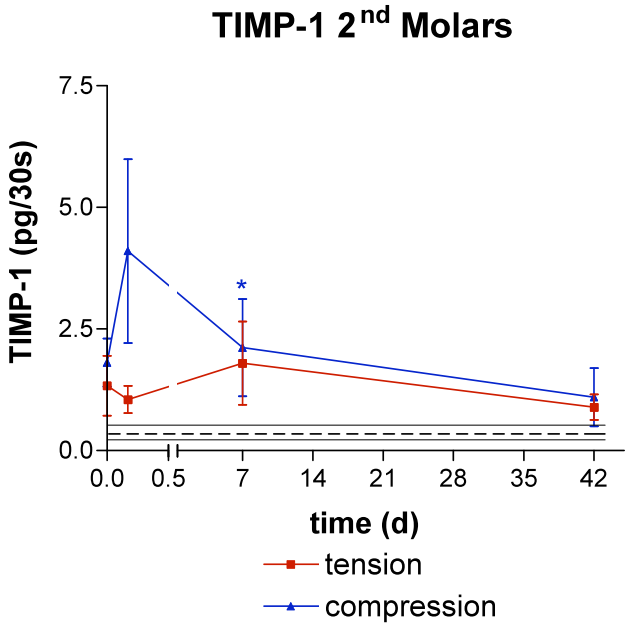


Figure 5.2A&B – TIMP-1 levels (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

5.3.1 Associations between clinical indices and TIMP-1 levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	*	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

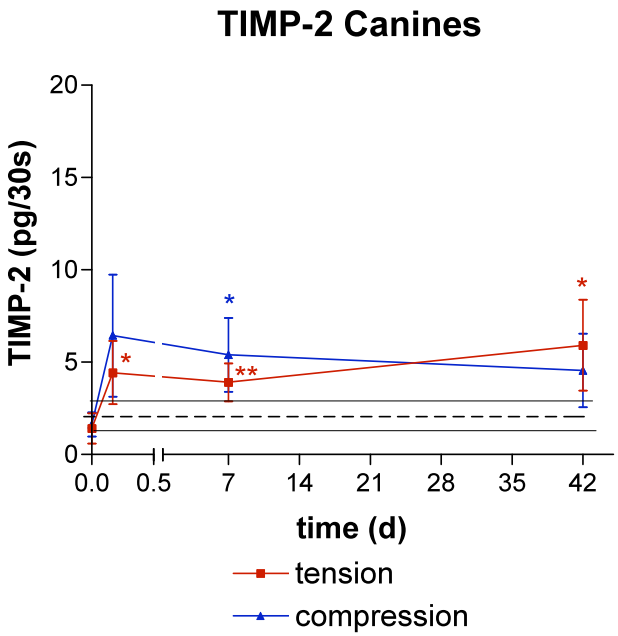
Table 5.2 Correlations between TIMP-1, plaque and bleeding indicies and the rate of tooth movement. . TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

5.4 Tissue Inhibitor of Metalloproteinases-2

There were significant differences between the levels of TIMP-2 expressed mesiobuccally to the upper canine, between time points Baseline and T2 ($p<0.05$), Baseline and T3 ($P<0.01$), Baseline and T4 ($P<0.05$) and T1 and T3 ($P<0.05$) (Figure 5.3A). There were also significant differences between the levels of TIMP-2 expressed distopalatally to the upper canine between Baseline and T3 ($p<0.05$) (Figure 5.3A). There were no significant changes in TIMP-2 levels at the second molars.

There were no significant differences in TIMP-2 expression between the mesiobuccal and distopalatal aspects of the canines or second molars at any of the time points (Figure 5.3B).

A



B

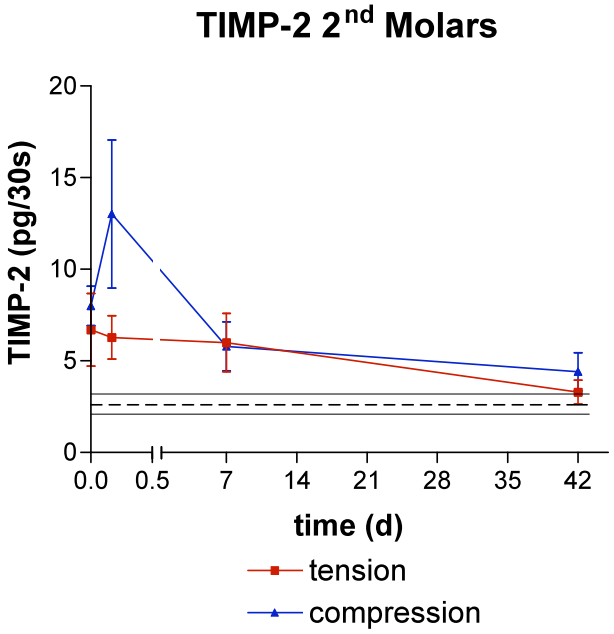


Figure 5.3A&B – TIMP-2 levels (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

5.4.1 Associations between clinical indices and TIMP-2 levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	*	ns
Canine CS	ns	*	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

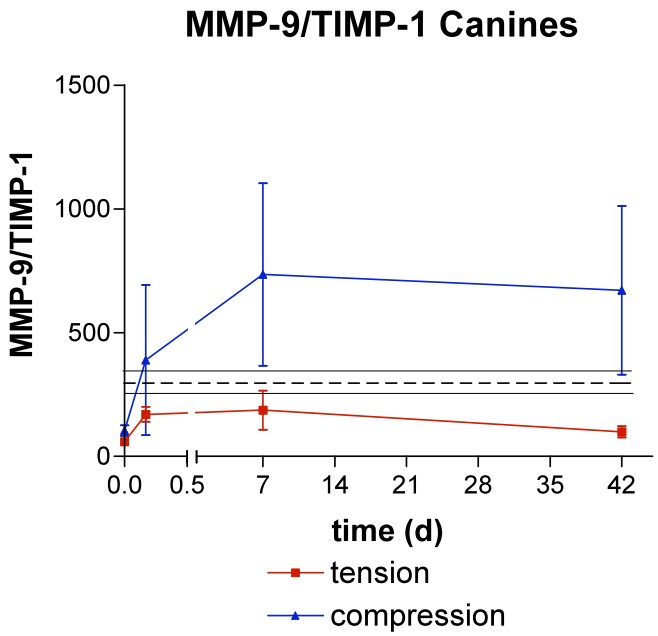
Table 5.3 Correlations between TIMP-2, plaque and bleeding indicies and the rate of tooth movement. . TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

5.5 Ratio MMP-9 / TIMP-1

There were no significant differences between the ratio of MMP-9/TIMP-1 expressed mesiobuccally or distopalatally to the upper canine or second molar, between any time points.

There were no statistically significant differences in the ratio of MMP-9/TIMP-1 between the mesiobuccal and distopalatal aspects of the canines or second molars at any time.

A



B

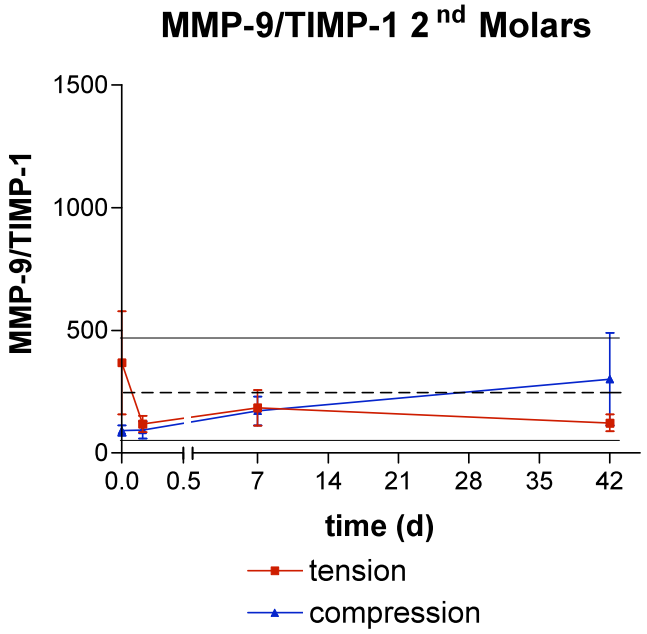


Figure 5.4A&B – MMP-9/TIMP-1 levels (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

5.5.1 Associations between clinical indices and MMP-9/TIMP-1 levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	*	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

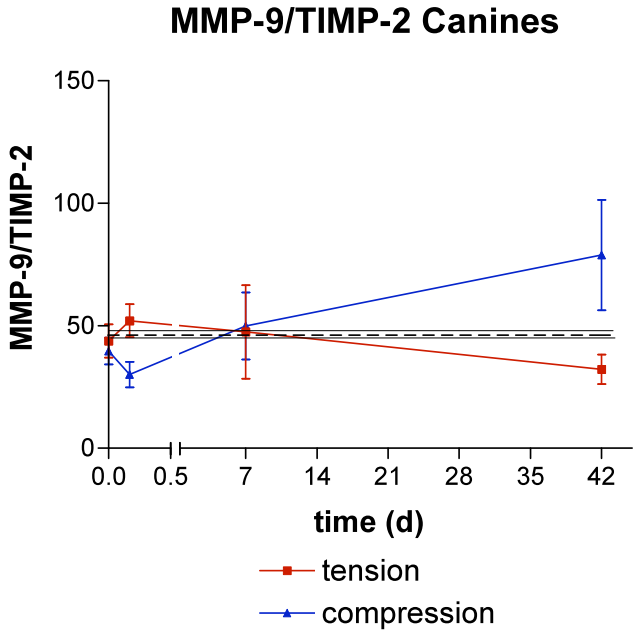
Table 5.4 Correlations between MMP-9/TIMP-1 ratio, plaque and bleeding indicies and the rate of tooth movement. . TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

5.6 Ratio MMP-9 / TIMP-2

There were no significant differences between the ratio of MMP-9/TIMP-2 expressed mesiobuccally or distopalatally to the upper canine or second molar, at any time (Figures 5.5A and 5.5B).

There were no significant differences in the ratio of MMP-9/TIMP-2 between the mesiobuccal and distopalatal aspects of the canines or second molars at any time.

A



B

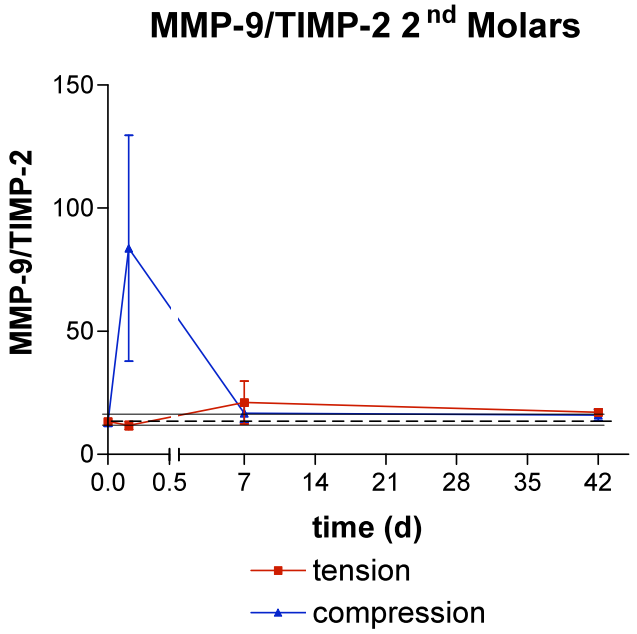


Figure 5.5A&B – MMP-9/TIMP-2 levels (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

5.6.1 Associations between clinical indices and MMP-9/TIMP-2 levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	*	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

Table 5.5 Correlations between MMP-9/TIMP-2 ratio, plaque and bleeding indicies and the rate of tooth movement. . TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

Chapter 6

Results

Bone Metabolites

6.1 Introduction

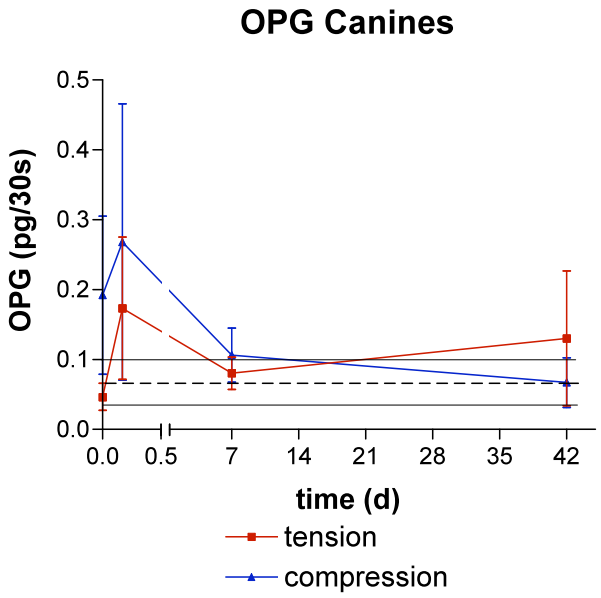
This chapter presents findings for bone metabolites measured in GCF taken at all time points during the orthodontic treatment. OPG and RANKL were assayed and are reported together with their ratios. Correlations between OPG and RANKL levels and clinical measures were analysed by Spearman Rank Correlations, unless otherwise stated.

6.2 Osteoprotegerin

There were no significant differences between the levels of OPG expressed mesiobuccally or distopalatally to the upper canine or at second molar sites between any time points (Figure 6.1A&B).

There were no statistically significant differences in the amount of OPG between the mesiobuccal and distopalatal aspects of the canines or second molars at any time.

A



B

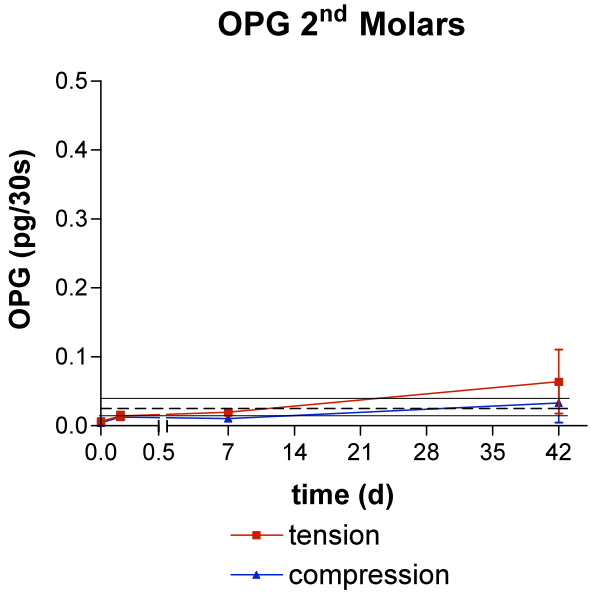


Figure 6.1A&B – OPG levels (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

6.2.1 Associations between clinical indices and OPG levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	*	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	*	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

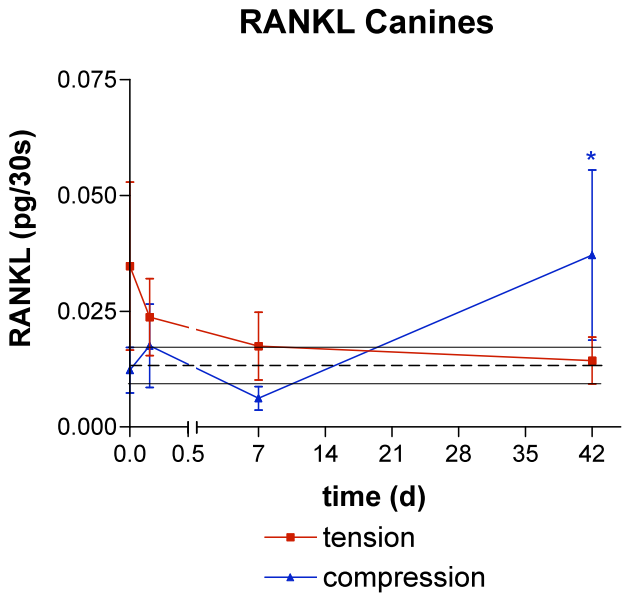
Table 6.1 Correlations between OPG, plaque and bleeding indicies and the rate of tooth movement. . TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

6.3 Receptor Activator of Nuclear Factor Kappa-B Ligand

There were no significant differences between the levels of RANKL mesiobuccally to the upper canine, between any of the time points. There was a significant difference between the levels of RANKL expressed distopalatally to the upper canine, between Baseline and T4 ($p<0.05$) (Figure 6.2A). There were no significant changes in the levels of RANKL at either second molar site (Figure 6.2B).

There were no statistically significant differences in the amount of RANKL between the mesiobuccal and distopalatal aspects of the canines or second molars at any time.

A



B

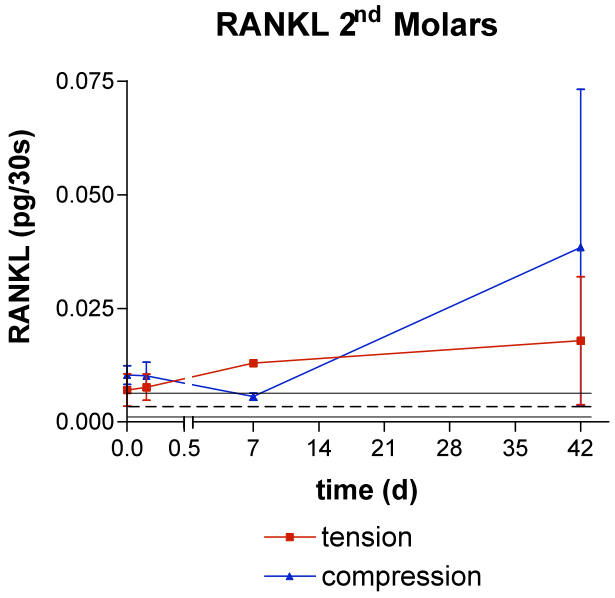


Figure 6.2A&B – RANKL levels (+/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

6.3.1 Associations between clinical indices and RANKL levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	*	*
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	*	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

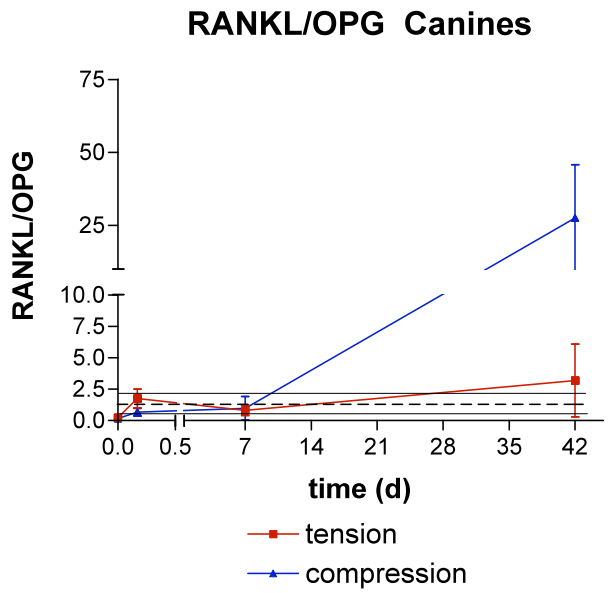
Table 6.2 Correlations between RANKL, plaque and bleeding indicies and the rate of tooth movement. . TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

6.4 RANKL/OPG Ratio

There were no significant differences between the RANKL/OPG ratio expressed mesiobuccally to the upper canine, between any time points. There was a significant difference between the levels of RANKL/OPG expressed distopalatally to the upper canine between T2 and T4 only ($p<0.05$). None of the changes at either second molar site were statistically different (Figure 6.3A).

There were no statistically significant differences in the amount of RANKL/OPG ratio expressed at any time (Figure 6.3B).

A



B

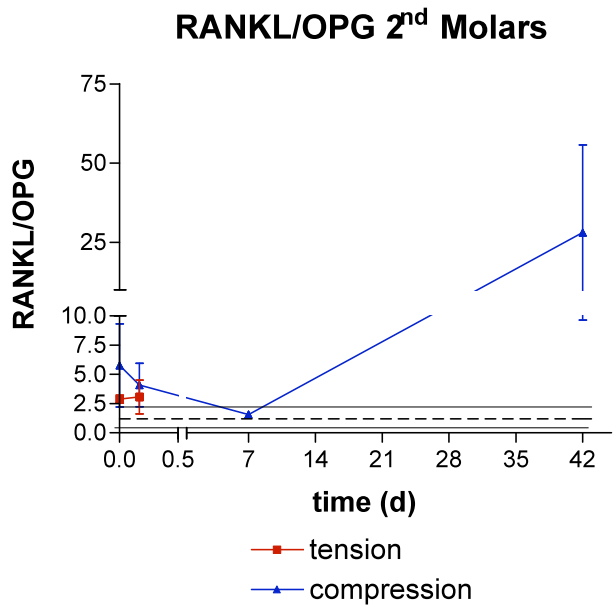


Figure 6.3A&B – RANKL/OPG ratio (mean +/- SEM), for sites of tension (red) and compression (blue) from maxillary canines and second molars. ***Dotted lines represent mean values at baseline and parallel solid lines represent the SEM of the baseline values. Statistical analysis by Kruskal-Wallis: *p<0.05; **p<0.01; ***p<0.001.***

6.4.1 Associations between clinical indices and RANKL/OPG levels.

	Correlations for each time point				
	Baseline	T1	T2	T3	T4
Plaque					
Canine TS	ns	ns	ns	*	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Bleeding					
Canine TS	ns	ns	ns	ns	ns
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns
Rate of Movement					
Canine TS	ns	ns	ns	ns	*
Canine CS	ns	ns	ns	ns	ns
Second molar TS	ns	ns	ns	ns	ns
Second molar CS	ns	ns	ns	ns	ns

Table 6.3 Correlations between RANKL/OPG ratio, plaque and bleeding indicies and the rate of tooth movement. . TS=Tension Sites CS=Compression Sites *=significant, B=borderline significance, ns=no significance.

Chapter 7

Discussion

7.1 Discussion

This study is the first to measure a large number of inflammatory, tissue and bone metabolism biomarkers using GCF from teeth undergoing orthodontic tooth movement. The development of protein/cytokine arrays has allowed testing of more than one biomarker within a small GCF sample, and thus profile changes occurring within the tissues surrounding the tooth.

In the present study three null hypotheses were tested; 1) There will be no change in the biomarker profile in GCF, 2) There will be no difference in the GCF biomarker profiles at compression and tension sites. 3) There will be no correlations between the various cytokines tested and the rate of tooth movement. Each of these hypotheses will be commented on in turn.

Previous studies that have recorded plaque and gingival health measurements have not reported them in detail (Yamaguchi et al., 2006; Giannopoulou et al., 2006). Unless high levels of oral hygiene are achieved prior to, and maintained during, treatment it is impossible to tell if the changes to the bio-markers are due to tooth movement or changes in oral hygiene and the resultant periodontal inflammation.

This study demonstrated significant increases in GCF volume at both the tension and compression sites on canines during movement. No differences in GCF volume were found between tension and compression sites and there were no differences between the GCF levels at the canines in comparison to the second molars. These findings suggest that there was a systemic response caused by the appliance placement and tooth movement, since a localised response would have lead to raised GCF around

the upper canines but unchanged GCF levels at the second molars which have no appliances or active forces acting upon them.

No significant differences were seen in full mouth plaque scores at any time. This demonstrated no compromise in plaque removal due to appliance placement. Neither did plaque levels increase when a distalising spring was placed, even though the springs harboured food particles.

Plaque scores at tension sites were significantly greater at the canines than the second molars at all time points except baseline. It is possible that this demonstrates that a combination of appliance placement and space opening mesial to the upper canine tooth may have encouraged food accumulation and hampered efficient plaque removal. Alternatively as the canine was the tooth undergoing most tooth movement it may be that it was more uncomfortable to clean.

Yamaguchi et al., (2006) found that Substance P and IL-1β were significantly increased in gingivae adjacent to teeth, which were orthodontically moved for up to 72 hours after force application, but remained at baseline levels around the control teeth. The forces applied to these teeth were probably not acting at their later sampling times, Substance P would have likely been increased at these time points also. Erdinc and Dincer (2004) suggested that from the results of their study that they felt that the perception of pain may be related to Substance P release. Hence teeth undergoing active movement are associated with pain. Substance P was not measured in the present study however other studies have also related increased IL-1β levels with increased pain perception (Giannopoulou et al., 2006**)**. IL-1β was shown to be

increased in canine tension sites in the present study at all time points and so that pain/discomfort may be the reason for increased plaque accumulation at these sites relative to the second molar.

Bleeding indices remained constant except for the decrease seen between baseline and 4 hours after a distalising force was applied, indicating that periodontal inflammation was less once the spring had been applied and then returned back to normal levels for the subject. Or perhaps that the forces created caused a relative tissue ischemia due to the distortion of periodontal cappiliaries and blood vessels.

Correlations between plaque scores and GCF levels were significant at both tension and compression canine sites 3 months after appliance placement. This suggests that before the distalising force was applied to the canine, increases in GCF associated with these teeth were due to inflammation from insufficient plaque removal.

Bleeding indices at tension sites were higher at the canines than the second molars after 4 hours and 7 days of distalising force. It is likely that active force causes more periodontal inflammation than passive force at a tension site.

Analysis of the amount of tooth movement showed that teeth had begun to move 4 hours after a distalising force had been applied and that they had moved a significantly greater distances 42 days after force application. Analysis of speed of movement demonstrated that 4hrs after the application of a distalising force tooth movement was fastest. It then decelerated over 7 and 42 days, perhaps illustrating that at 4 hours the tooth was in the first phase of tooth movement in displacement

(Burstone, 1962). During the second phase of tooth movement which is a lag period with relatively low rates of tooth displacement. Hyalinization occurs in the PDL in areas of compression and no further tooth movement can happen until necrotic tissue is removed.

Analysis of cytokines around the canines revealed significant increase in IL-1β, IL-8, and TNFα in comparison to baseline levels at tension sites once a distalising force was active. 4 hours after the distalising force was placed IL-1β levels peaked at canine tension and compression sites and IL-8 levels peaked at canine tension sites. This suggests that these levels rose quickly in response to the orthodontic force as part of an acute reaction. IL-1β attracts leukocytes and stimulating fibroblasts and endothelial cells osteoclasts which promote bone resorption and inhibit bone formation. IL-8 is thought to be a trigger factor for bone remodelling TNFα which promotes bone resorption, it is therefore likely that both should peak at tension sites. IL-8 also regulates the influx of leukocytes to the site of inflammation. Whether this reaction is inflammatory or not is a subject for debate. Meikle (2006) suggested that tooth movement did not meet three of the four classical criteria of inflammation (redness, heat, swelling and pain), only pain was present. He suggested that tooth movement was best regarded as an exaggerated form of normal physiological turnover combined with foci of tissue repair. TNF alpha levels peaked 42 days after a distalising force was applied, perhaps revealing a more chronic inflammatory response. IL6 showed an increase at the tension site 3 months into appliance treatment and 42 days after the distalising force had been applied, suggesting that it was released more slowly and not as a response to acute inflammation. GM-CSF and IFNγ levels remained constant at canine sites. TNFα and GM-CSF levels rose at the

compression sites on the second molars 3 months after appliance placement. Four hours after the distalising force was applied the TNFα values rose again. All other cytokine levels tested at the second molars remained constant. Increases in cytokines recorded at the control second molar teeth, may imply that the application of force affects the whole mouth, though this change was more transient than that seen at the canines.

At Baseline and three months into treatment IL-1β , IL-8, TNFα, IFNγ levels all showed associations with plaque levels at canine sites. There were no significant correlations between plaque and bleeding indices and the levels of IL-1β , IL-8 or TNFα once the distalising force had been applied. This suggests subsequent changes in IL-1β , IL-8 and TNFα were due to orthodontic force and tooth movement. IL-6 and GM-CSF were not associated with plaque and bleeding scores at any point in the study. This suggests fluctuations in cytokine levels once a distalising force was applied were due to inflammation resulting from orthodontic forces.

This study agrees with clinical findings from other studies which have shown that IL-1β and TNFα are all elevated in gingival crevicular fluid collected from patients during the early phases of orthodontic tooth movement (Grieve et al 1994 ; Lowney et al 1995 ; Uematsu et al. 1996).

Dudic et al (2006), found that the GCF levels of IL-1β, SP, and PGE2 in orthodontically moved teeth were significantly higher than for the control teeth at both tension and compression sides throughout force application. The increase, relative to baseline values, was generally higher in tension sides. They also found

that although biomarker patterns were different at tension and compression sites, with generally higher values of cytokines at tension site, they were not significantly raised. The present findings support this view.

Tissue metabolites showed similarities to cytokines with MMP-9, TIMP-1 and TIMP-2 expressed more at canine tension sites following application of distalising force, with peaks at 7 days for TIMP-1 and 42 days for TIMP-2. MMP-9 was low after 7 days. Increases in MMP-9 occurred 7 days after force application at the compression sites. Initially it seems that the levels of all bone metabolites increased in response to the orthodontic distalising force. But that by 7 days the TIMPs 1 and 2 remained high at tension sites, whereas MMP-9 levels dropped at the tension sites and increased at the compression sites. It is thus likely that new bone formation was at progress in the tension sites by this time. The higher levels of TIMPs would have inhibited the release of MMP-9, reducing its level so that boney resorption was occurring at compression sites. The ratios of MMP-9 to TIMP-1 and TIMP-2 did not produce significant conclusions.

There were no correlations between increases in plaque and bleeding scores and any tissue metabolites once a distalising force was applied. It can thus be deduced that changes in biomarker levels were due to the distalising force and resultant tooth movement.

Regarding the bone metabolites, none of the changes in OPG levels were significant. However RANKL levels were significantly higher after 42 days at the canine compression sites. Also the ratio of OPG to RANKL was significantly different after

42 days. This suggests that the OPG RANKL interaction occurs later on in tooth movement. There were no correlations with plaque and bleeding scores at compression sites so these changes are most likely be a consequence of applied force and tooth movement. Kanzaki et al. (2002) found that culture media from compressed PDL fibroblasts stimulated osteoclastogenesis in peripheral blood mononuclear cell cultures and that RANKL mRNA expression was upregulated in the cells. OPG expression in contrast remained unchanged as was found in this study. Kawasaki et al, (2006) discussed the differences between adult and juveniles for the expression of OPG and RANKL in GCF. The amount of tooth movement for juveniles was larger than for adults after 168 hours (7 days). After 24 hours RANKL levels were increased and those of OPG decreased in GCF samples from the compression side during orthodontic tooth movement in both juveniles and adults. The RANKL/OPG ratio in GCF from adult patients was lower than that in the juvenile samples perhaps explaining why tooth movement is slower in adults.

The ratio of OPG to RANKL around second molar compression sites was similar to that at canine compression sites, suggesting that bony resorption was occurring at both of these sites simultaneously.

Yamaguchi et al. (2006) reported on associations between increases in RANKL expression in compressed periodontal ligament cells and root resorption. Compressed PDL cells obtained from patients demonstrating severe external apical root resorption produced large amounts of RANKL, small amounts of OPG and stimulated osteoclast formation. A mini review of the topic Tyrovola et al. (2008) also described a link between RANKL levels, osteoclastic activation and root resorption. If an optimal

RANKL level could be targeted it might be possible to create tooth movement without causing resorption.

Increases in TNFα, GM-CSF, IL-1β and IL-8 at the canines were correlated to speed of movement, indicating a rapid inflammatory response to applied force. Tissue and bone metabolism biomarkers followed different patterns. MMP-9, TIMP-2 and TIMP-1 correlated with speed of movement 4 hours after application of a distilasing force. However, MMP-9 levels continued to increase at sites of compression and TIMP-1 levels continued to increase at sites of tension 7 days after force. These changes in MMP-9 would be consistent with a need to decrease the density of soft tissue under compression. Bone metabolism biomarkers were not significantly correlated with speed of movement; however the greatest changes in OPG levels were seen within 4 hours of force application. RANKL increased at 42 days after application of a distilising force. This could be the start of Phase 3 of tooth movement, where the increase in RANKL at sites of compression could indicate that activation of osteoclasts and bone resorption has begun.

The present study subjects were divided into 3 groups depending on their overall rate of tooth movement. Biomarker profiles were then compared for each of the groups to see if there were any obvious differences between fast movers, medium movers and slow movers. However, no statistically significant differences were found and only IL-1β showed a clear pattern with levels being highest in fast movers at all times and lowest in slow movers.

The present study has provided greater insight into tooth movement and the biological response of the surrounding tissues to this movement. However the group size was insufficient to give the study enough power to demonstrate possible significant differences. Post Hoc power analysis suggests that a sample size greater than 120 patients would be required, and the subject warrants further. It would also be interesting to add an additional time point at 84 days to look at the changes occurring once the tooth is in Stage 3 of orthodontic tooth movement (Burstone, 1962).

Much of the recent research into tooth movement has looked at ways to reduce the lag phase of tooth movement, when hyalinisation occurs. Studies have looked at minimising hyalinisation by examining the amount and duration of force on the teeth. It would be useful to look at a profile of cytokines in subjects receiving differing amounts of force and their durations.

Chapter 8

Conclusions

8.1 Conclusions

This study investigated the effects of orthodontic tooth movement on the periodontium by analysing cytokine, tissue and bone metabolite levels within GCF. As plaque and bleeding scores remained constant after force application, it is likely that biomarker changes in the periodontium most likely resulted from orthodontic forces.

The null hypotheses being tested in this study were that, when an orthodontic force applied to a tooth

1) There will be no change in the biomarker profile in GCF.

This was disproved and this null hypothesis is rejected. There were significant changes in the profile of several biomarkers at various times which were consistant with tissue remodelling.

2) ***There will be no difference in the GCF biomarker profiles at the compression and tension sites.***

Although differences were seen between tension and compression sites, none were statistically significant. It is likely that a greater number of subjects are needed to prove statistical significance between sites. Thus this hypothesis stands.

3) ***There will be no correlations between the various cytokines tested and the rate of tooth movement.***

This was also disproved by increases of TNFα, GM-CSF, IL-1β and IL-8 at the canines, which correlated with the rate of tooth movement. MMP-9, TIMP-2 and TIMP-1 levels increased in line with speed of movement 4 hours after application of force.

Chapter 9

References

9.1 References

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Appendices

Appendix A 1

Participant Information Sheet

Orthodontic tooth movement

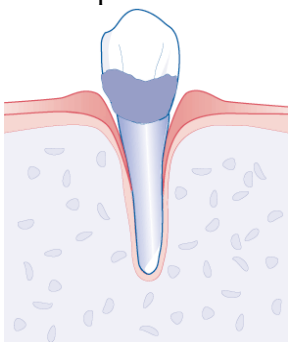
Version 4.0 (18th June 2008)

Assessment of changes in gingival fluid biomarkers during orthodontic tooth movement

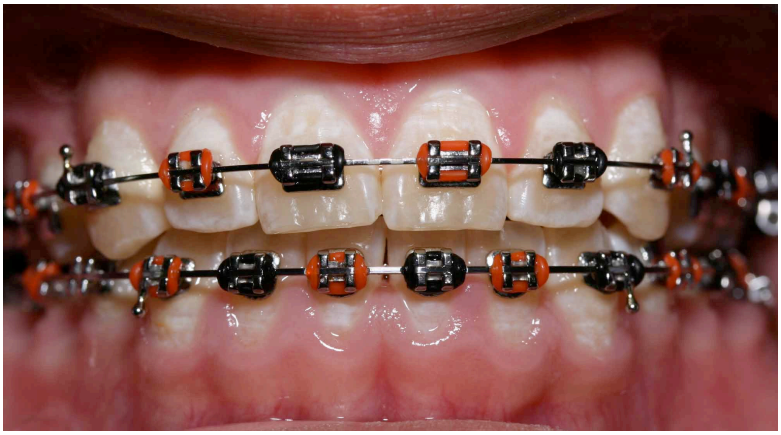
You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with your parents and others if you wish. Ask us if there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

What is the purpose of the study?

By measuring the forces applied to a tooth and the concentration of certain chemicals released by the tissues around a tooth, this study aims to assess whether the levels of such chemicals change when different forces are applied. This study will help to create a better understanding of the bone processes that take place when a force is applied to a tooth. It is hoped that it will ultimately help to identify those patients whose teeth will move faster or slower, so that treatment planning may be improved in the future to improve results for individual patients.



Why have I been chosen to take part in the study?
You have been chosen to take part in the study because you are a patient aged 12-16 years, with good general health and are about to undergo a course of treatment with fixed braces.



Do I have to take part in the study?
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to stop at any time and without giving a reason. A decision to stop at any time, or a decision not to take part, will not affect the care you receive.

What will happen to me if I take part?
If you decide to take part in this study you will be treated in exactly the same manner as all our patients and will receive your treatment as normal but will be asked to attend the hospital for one long visit and one extra appointment to enable some samples to be taken from you and samples will also be taken at four of your routine appointments. Also you will have impressions taken of your teeth as well as gum health and plaque measurements at every appointment when samples are taken.

You **will not** be eligible to take part in the study if-

- You are a smoker.
- Have poor oral hygiene
- Signs of significant gum disease.
- Evidence of bone loss around the teeth on xrays.
- Have taken a courses of anti-inflammatory's or antimicrobial medicines within 1 month prior to the study.

What do I have to do?
At your treatment planning appointment (baseline) the study will be discussed, you may ask any questions that you need to and your consent will be obtained and a medical history taken. Your gum health will be assessed and the amount of plaque on your teeth will be measured. Paper strips will be used to collect fluid from around your teeth (called gingival crevicular fluid or GCF) this is painless and you will not feel the sample collection. Three months later the samples will be taken again and a spring will be placed to help to close the spaces where teeth have been taken out (as per normal) your gum health and plaque levels will again be assessed and an impression taken of your top teeth. Four hours later the samples will be repeated, together with gum health, plaque levels and an impression. Four days later you will need to attend an extra visit for samples to be taken, your gum health and plaque levels will be assessed and an impression of the top teeth taken. Five weeks later at your routine brace adjustment appointment more samples will be taken, gum health and plaque levels will be assessed and an impression of the top teeth taken. Treatment will then continue as normal.

Will my taking part in the study be confidential?
You will be allocated a study number, so that your name will not be disclosed to anyone except the people treating you. Your name will not be identified with the data collected other than by a code. If you wish to know your personal results these can be shared with you after the study is complete. You will have a dedicated team looking after you who will answer any questions you have at any stage.

Are there any side effects of the treatment?
You will be receiving no additional treatment as part of the study. There are no side effects involved in giving the samples as previously discussed.

What are the possible disadvantages and risks of taking part?
There are no disadvantages to taking part, you will have long visit and one extra visit of about 10 minutes and you will need the gum fluid samples taken at this visit. At the longer visit you are free to leave the hospital after the morning part of your appointment, but we need you to return 4 hours later for the afternoon part.

What are the possible benefits of taking part?
You will get the chance to have your brace checked at the additional appointment, which may help to address any problems or concerns. Also patients in the study will have their oral hygiene levels very closely monitored, which will minimise their risk of developing early decay around the brace/gum disease.

What if new information becomes available?
If new information becomes available, you will be told at your next visit.

What happens when the research study stops?
At the end of the study, you will return to normal treatment.

What if something goes wrong?
You are only providing gum fluid samples, so nothing will go wrong with this aspect of the study. However, if you have any worries or concerns or problems a member of the study team will be available to discuss these with you. If you wish to make a complaint you should contact Professor P Lumley by writing to the Birmingham Dental Hospital, St Chad's Queensway, B4 6NN or calling 0121 236 8611.

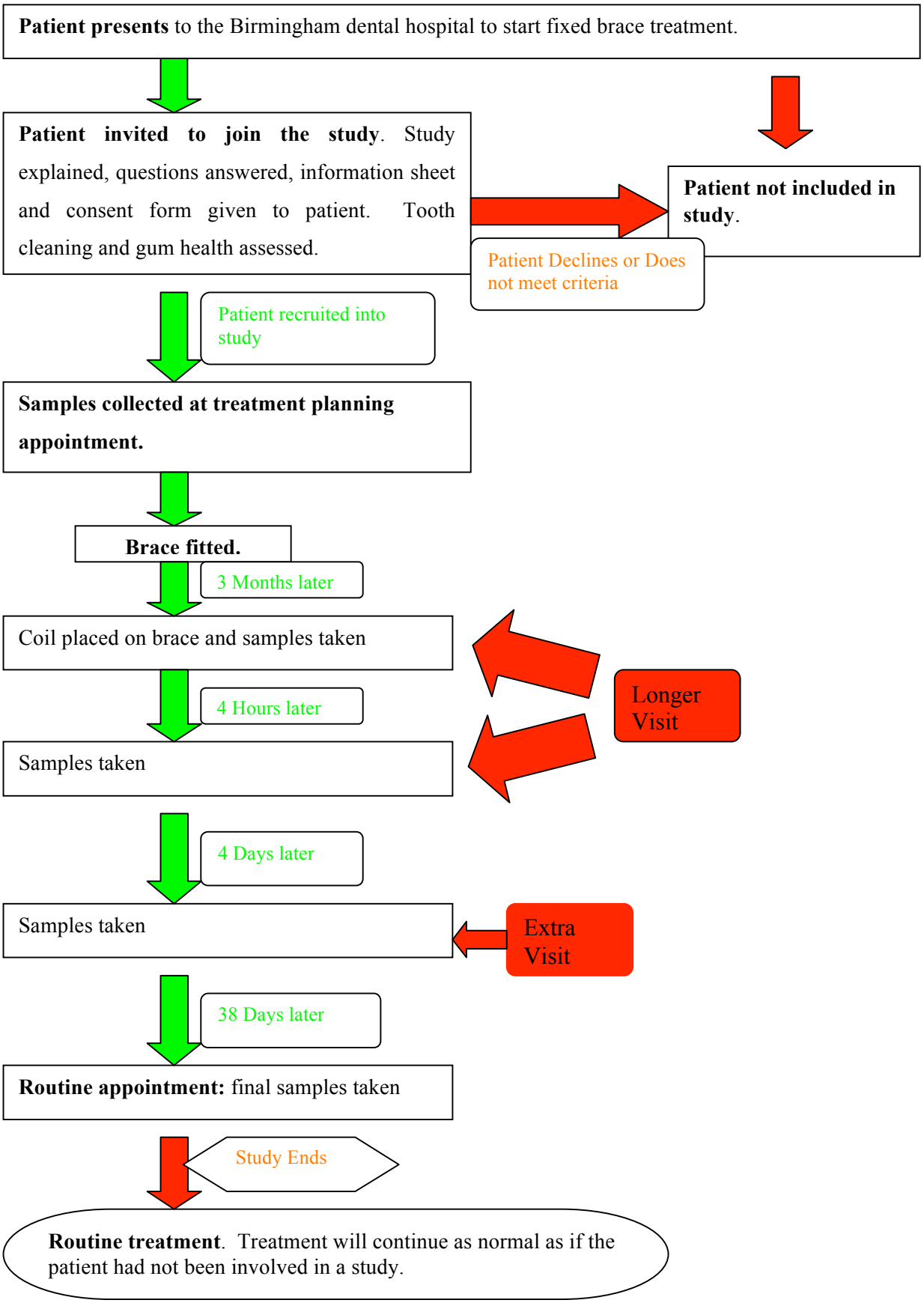
Who is organising and funding the research?
This study is one that we have designed and led at the Dental Hospital.

What about my travel costs for the additional visit?
If you request re-imbursement of reasonable travel costs for the additional visit this will be provided in cash upon presentation of receipts. Please ask the study clinician and they will arrange this for you.

Who has reviewed this study?
The study has been reviewed by Coventry Research Ethics Committee.

Contact for further information?
If you need further information please phone 0121 237 2735 and ask to speak to Jay Wilson who is managing the study.

Study Flow Chart



Participant Consent Sheet

Version 4.0 (18th June 2008)

Assessment of changes in gingival fluid biomarkers during orthodontic tooth movement

Consent Statement:
I have read the information sheet (version 4.0 dated 18th June 2008) and been given the opportunity to ask any questions I wished to ask. I understand what is involved in the project and I agree to take part in the study. I understand that the study team will need to access my treatment notes and that anonymous data will need to be analysed by members of the study team. I understand that my identity will be confidential to the study team alone and that I may withdraw from the study at any stage, without detriment to my treatment.

Subject signature & date

Subject Name (please print)

Name & Signature & date of team member taking consent

Parent Information Sheet

Orthodontic tooth movement

Version 4.0 (18Th June 2008)

Assessment of changes in gingival fluid biomarkers during orthodontic tooth movement

Your child is being invited to take part in a research study. Before you decide 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. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?
By measuring the forces applied to a tooth and the concentration of certain chemicals released by the tissues around a tooth, this study aims to assess whether the levels of such chemicals change when different forces are applied. This study will help to create a better understanding of the biological processes that take place when a force is applied to a tooth. It is hoped that it will ultimately help to identify those patients whose teeth will move faster or slower, so that treatment planning may be improved in the future to optimise results for individual patients.

Why has my chid been chosen to take part in the study?
Your child has been chosen to take part in the study because he/she is a patient aged 12-16 years, with good general health and is about to undergo a course of fixed brace treatment.

Does my child have to take part in the study?
It is up to you and your child to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and both be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care received by your child.

What will happen to my child if we take part?
If you decide to take part in this study your child will be treated in exactly the same manner as all our patients and will receive their treatment as normal but will be asked to attend the hospital for one long visit and one extra appointment to enable some samples to be taken, samples will also be taken at four routine appointments. Also impressions will be taken of your child's teeth as well as gum health and plaque measurements at every appointment when samples are taken.

Your child **will not** be eligible to take part in the study if-

- They are a smoker.
- Have poor oral hygiene
- Signs of signifficant gum disease.
- Evidence of bone loss around the teeth on xrays.
- Have taken a course of anti-inflammatory's or antimicrobial medicines within 1 month prior to the study.

What do we have to do?
At the treatment planning appointment (baseline) the study will be discussed, you may ask any questions that you need to and your consent will be obtained together with your child's and a medical history taken. Gum health will be assessed and the amount of plaque on your child's teeth will be measured. Paper strips will be used to collect fluid from around the teeth (called gingival crevicular fluid or GCF) this is painless and your child will not feel the sample collection. Three months later the samples will be taken again and a spring will be placed to help to close the spaces where teeth have been taken out (as per normal) gum health and plaque levels will again be assessed and an impression taken of the top teeth. Four hours later the samples will be repeated, together with gum health, plaque levels and an impression. Four days later you will need to attend an extra visit for samples to be taken, gum health and plaque levels will be assessed and an impression of the top teeth taken. Five weeks later at the routine brace adjustment appointment more samples will be taken, gum health and plaque levels will be assessed and an impression of the top teeth taken. Treatment will then continue as normal.

Will our taking part in the study be confidential?
Your child will be allocated a study number, so that their name will not be disclosed to anyone except the clinical people treating you. Their name will not be identified with the data collected other than by a code. If you wish to know your child's personal results these can be shared with you after the study is complete. You will have a dedicated team looking after you who will answer any questions you have at any stage.

Are there any side effects of the treatment?
Your child will be receiving no additional treatment as part of the study. There are no side effects involved in giving the samples as previously discussed.

What are the possible disadvantages and risks of taking part?
There are no disadvantages to taking part, you will have one long visit and one extra visit of about 10 minutes, you will need the gum fluid samples taken at this visit. At the longer visit you and your child are free to leave the hospital after the morning part of your appointment, but we need you to return 4 hours later for the afternoon part.

What are the possible benefits of taking part?
Your chid will get the chance to have their brace checked at the additional appointment, which may help to address any problems or concerns. Also patients in the study will have their oral hygiene levels very closely monitored, which will minimise their risk of developing early decay around the brace/gum disease.

What if new information becomes available?
If new information becomes available, you will be informed of this at your next visit.

What happens when the research study stops?
At the end of the study, your child will enter a normal review process and any further treatment needed will be arranged with a member of staff as is normal practice.

What if something goes wrong?
Your child is only providing gum fluid samples, so nothing will go wrong with this aspect of the study. However, if you have any worries or concerns or problems a member of the study team will be available to discuss these with you. If your child is harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. If you wish to make a complaint you should contact Professor P Lumley by writing to the Birmingham Dental Hospital, St Chad's Queensway, B4 6NN or calling 0121 236 8611.

Who is organising and funding the research?
This study is one that we have designed and led at the Dental Hospital.

What about my travel costs for the additional visit?
If you request re-imbursement of reasonable travel costs for the additional visit this will be provided in cash upon presentation of receipts. Please ask the study clinician and they will arrange this for you.

Who has reviewed this study?
The study has been reviewed by Coventry Research Ethics Committee.

Contact for further information?
If you need further information please phone 0121 237 2735 and ask to speak to Jay Wilson who is managing the study.

Parent Consent Sheet

Version 4.0 (18th June 2008)

Assessment of changes in gingival fluid biomarkers during orthodontic tooth movement

Childs Name:…….

Consent Statement:
I have read the information sheet (version 4.0 dated 18th June 2008), and been given the opportunity to ask any questions I wished to ask. I understand what is involved in the project and I agree for my child to take part in the study. I understand that the study team will need to access my child's treatment notes and that anonymous data will need to be analysed by members of the study team. I understand that my child's identity will be confidential to the study team alone and that we may withdraw from the study at any stage, without detriment to treatment.

Guardian signature

Guardians Name (Please print) & Date

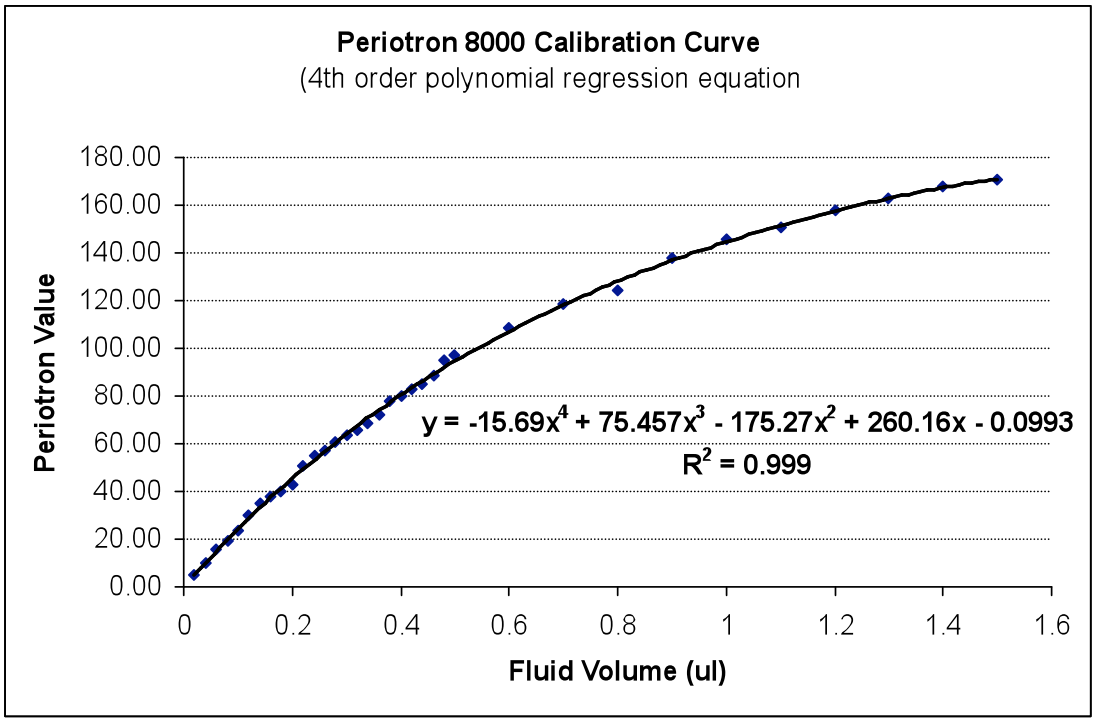
Name & Signature & date of team member taking consent

Appendix B 1

Periotron 8000 calibration.

Raw data

Volume (ul)		Periotron Reading		Mean
	1	2	3	
0.02	4	5	5	4.67
0.04	9	10	10	9.67
0.06	15	16	16	15.67
0.08	19	19	19	19.00
0.1	23	24	23	23.33
0.12	29	32	30	30.33
0.14	34	35	36	35.00
0.16	37	38	39	38.00
0.18	40	39	41	40.00
0.2	43	42	44	43.00
0.22	50	51	52	51.00
0.24	55	55	55	55.00
0.26	57	58	57	57.33
0.28	60	62	61	61.00
0.3	64	63	64	63.67
0.32	65	66	66	65.67
0.34	68	68	69	68.33
0.36	72	72	73	72.33
0.38	77	78	78	77.67
0.4	80	81	80	80.33
0.42	83	83	83	83.00
0.44	86	85	85	85.33
0.46	88	89	88	88.33
0.48	95	96	95	95.33
0.5	98	97	97	97.33
0.6	107	110	109	108.67
0.7	119	118	118	118.33
0.8	123	124	125	124.00
0.9	139	137	137	137.67
1	146	145	146	145.67
1.1	152	151	150	151.00
1.2	159	158	156	157.67
1.3	164	163	161	162.67
1.4	169	168	167	168.00
1.5	171	172	169	170.67


Periotron 8000 Calibration Curve
(4th order polynomial regression equation
Periotron Value
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Fluid Volume (ul)
y = -15.69x4 + 75.457x3 - 175.27x2 + 260.16x - 0.0993
R2 = 0.999

Appendix B 2

Periotron values for set fluid volumes calculated from the previous equation.

Volume	Periotron value
0.004	1
0.008	2
0.012	3
0.016	4
0.02	5
0.024	6
0.028	7
0.032	8
0.036	9
0.04	10
0.044	11
0.048	12
0.052	13
0.056	14
0.06	15
0.065	16
0.069	17
0.073	18
0.077	19
0.082	20
0.086	21
0.09	22
0.095	23
0.099	24
0.103	25
0.108	26
0.112	27
0.117	28
0.121	29
0.126	30
0.13	31
0.135	32
0.14	33
0.144	34
0.149	35
0.154	36
0.158	37
0.163	38
0.168	39
0.173	40
0.178	41
0.183	42
0.188	43
0.192	44

Volume	Periotron value
0.277	60
0.282	61
0.288	62
0.294	63
0.299	64
0.305	65
0.311	66
0.317	67
0.323	68
0.329	69
0.335	70
0.341	71
0.347	72
0.353	73
0.359	74
0.366	75
0.372	76
0.378	77
0.385	78
0.391	79
0.398	80
0.404	81
0.411	82
0.417	83
0.424	84
0.431	85
0.438	86
0.445	87
0.452	88
0.459	89
0.466	90
0.473	91
0.48	92
0.488	93
0.495	94
0.503	95
0.511	96
0.518	97
0.525	98
0.533	99
0.541	100
0.549	101
0.557	102
0.565	103

Volume	Periotron value
0.707	119
0.717	120
0.727	121
0.737	122
0.747	123
0.757	124
0.768	125
0.778	126
0.788	127
0.799	128
0.81	129
0.821	130
0.832	131
0.844	132
0.856	133
0.867	134
0.879	135
0.89	136
0.903	137
0.915	138
0.927	139
0.94	140
0.953	141
0.966	142
0.979	143
0.992	144
1.006	145
1.02	146
1.034	147
1.048	148
1.063	149
1.078	150
1.093	151
1.108	152
1.124	153
1.14	154
1.157	155
1.173	156
1.19	157
1.208	158
1.226	159
1.244	160
1.263	161
1.283	162

0.197	45
0.202	46
0.208	47
0.213	48
0.218	49
0.223	50
0.228	51
0.233	52
0.239	53
0.244	54
0.249	55
0.255	56
0.26	57
0.266	58
0.271	59

0.573	104
0.581	105
0.59	106
0.599	107
0.607	108
0.615	109
0.624	110
0.633	111
0.642	112
0.651	113
0.66	114
0.669	115
0.679	116
0.688	117
0.698	118

1.303	163
1.323	164
1.345	165
1.368	166
1.391	167
1.416	168
1.442	169
1.47	170
1.499	171
1.532	172
1.569	173
1.614	174
1.673	175