CHAPTER 1

INTRODUCTION

Background and Rationale

In the orthodontic treatment, the tooth movement is necessary for the treatment especially in the extraction case. The orthodontists have attempted to find the materials and methods which can enhance the orthodontic tooth movement and shorten the treatment time. There are varieties of orthodontic material for canine retraction. They are different in magnitude and type of forces such as nickel-titanium closed coil spring (NiTi closed coil spring), elastic c-chain, active ligature, magnet, screw, etc. Among these orthodontic materials; NiTi closed coil spring, elastic c-chain and active ligature are commonly used. Therefore, the decision of best choice is necessary.

In recent years, it is recommended to use NiTi closed coil spring for canine retraction and incisor retraction in many systems such as Damon® system (self-ligating bracket). This kind of spring is constant force delivery and easy to use which are the advantages over other materials. However, NiTi close coil spring is an expensive material.[1] In contrast, elastic c-chains are unable to deliver a continuous force over a period of treatment.[2] Besides, elastic c-chains are affected by water, Coke®, turmeric solution and temperature while NiTi close coil springs are only affected by temperature.[3] Conversely, Han and Quick (1992) reported that the NiTi close coil springs are not affected by incubation in artificial saliva at 37 $^{\circ}$C.[4] Because of the force decayed property of elastic c-chain, they have to be changed every four weeks. Some orthodontists recommended that elastic c-chain, when placed, needed to extend twice their original length to compensate for the large force loss.[2] However, the elastic c-chain provides a cheaper treatment option than using NiTi closed coil spring.

There are many studies that compared the rate of space closure between using different materials. Sonis (1994) and Samuels (1998) showed that the NiTi closed coil springs gave the most rapid rate of space closure which were compared with active ligature and conventional elastic 3/16 inches.[5, 6] In contrast, Dixon *et al*. (2002) and Nightingale and Jones (2003) showed that the rates of space closure were not statistically significant difference between using NiTi close coil springs and elastic c-chains, although, they found that NiTi close coil springs gave higher mean rate of space closure than elastic c-chains.[7, 8] Therefore, there is controversy on this topic.

In recent years, there are many advanced researches that studying on cellular and molecular response to mechanical force. There are many studies reported on the osteoclastic bone resorption effects of biochemical mediators to the orthodontic tooth movement *in vitro*, in animal and in human.[9, 10] Therefore, the investigation of biochemical mediator secretion is useful for data base to determine the proper method and material for canine retraction.

When the forces exerted on the tooth crowns passed through the roots and transmitted to the surrounding tissue of the periodontium, the tooth was moved within the periodontal ligament (PDL) space and blood flow was changed. Some areas were compressed ligament with decreased blood flow while others were stretched ligament with increased blood flow in opposition areas. The distortions of PDL cells and extracellular matrices and alterations in blood flow result in the changes of the chemical environment and acute inflammatory response.[11] An acute inflammatory response characterized by periodontal vasodilation and the migration of inflammatory cells out of PDL capillaries. These inflammatory cells produce and release cytokines, also known as inflammatory mediators, into the gingival crevicular fluid (GCF).[9] These cytokines can either act directly or stimulate the release of other biologically active agents which subsequently, resulting in cellular differentiations and activities. For example, the osteoclast precursor cells would differentiate to osteoclasts or the osteoblast precursor cells would differentiate to osteoblasts. They result in both bone resorption and formation.[12] Local mediators related with bone resorption include interleukins (IL) 1,[9, 13] 6,[9, 10] 8,[14] tumor necrosis factor α (TNF-α),[9, 10] prostaglandin E (PGE)[15-18] and so on. While the mediators related with bone formation are transforming growth factor β (TGF-β),[9, 10] alkaline phosphatase[19] and etc.

Interleukins are one of the proinflammatory cytokines released from many cell types such as fibroblasts, osteoclasts, polymorphonuclear leukocytes (PMN), etc.[20] IL-1, IL-6 and IL-8 are proinflammatory interleukins that have been identified in GCF during orthodontic tooth movement.[9, 21] IL-8 is produced and secreted by many cells such as fibroblasts, epithelial cells, endothelial cells and alveolar macrophages, in response to inflammation.[20] It is a potent proinflammatory cytokine that plays an important role in the recruitment and activation of neutrophils during inflammation. Therefore, neutrophils and other inflammatory cells migrate out of PDL capillaries to the inflammatory region. These inflammatory cells produce and release IL-1β and so on. There are many researches which studied on IL-1β during orthodontic tooth movement.[22-24] Many investigators found that IL-1β can stimulate bone resorption and can affect to tooth movement. And also IL-8 was known as biochemical mediator of bone resorption. IL-8 levels were found to be positively correlated with IL-1β in GCF of periodontitis patients.[25] Additionally, there is little knowledge about IL-8 in orthodontic field. So, we chose to evaluate the IL-1β and IL-8 secretion in this study.

The aims of this research were to examine whether the inflammatory mediators: IL-1β and IL-8 were presented and changed in GCF of the orthodontic patient undergoing canine retraction (orthodontic tooth movement) when using two different kinds of material; NiTi closed coil spring and elastic c-chain. Data obtained from this study will be used to choose material of choice for patient in the future.

Review of Literature

Orthodontic treatment aims to correct malocclusion by moving teeth through the alveolar bone. Mechanical force exerted on the roots and transmitted to the periodontium initiates bone remodeling activity, in which bone is resorbed in the sites under pressure and deposited in the sites under tension. The mechanical stress exerted to the tissues leads to physical and biochemical responses (Fig.1).[26] However, the exact mechanism by which the stress orchestrates tooth movement is not clear.

In recent years, it is known that orthodontic forces stimulate proliferation and activity of osteoblasts and osteoclasts. The mechanical force acts as primary stimulus or first messenger altering cell activity through the plasma membrane.[26] The intramembranous components, calcium-ion and membrane-bound enzymes, then elevate cyclic nucleotide molecules, such as cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP).[26] The cyclic nucleotides act as second messengers, in that they convert the force at the cell membrane into a cellular response.[26]

Many investigators found that application of force to the bone generated electric potentials (piezoelectric response) and used of external electricity enhanced osteogenesis.[27-29] Davidovitch *et al.* (1980) showed that electric currents applied to periodontal tissues (PDL) increased the levels of cAMP and cGMP in alveolar bone, osteoblasts and PDL cells, in addition, combined electric orthodontic treatment accelerated tooth movement.[30, 31]

In orthodontics, the early phase of tooth movement involved an acute inflammatory response which is characterized by migration of inflammatory cells from dilated PDL capillaries.[13] The inflammatory cells release cytokines, also known as inflammatory mediators, such as interleukins (IL), prostaglandin (PG) and tumor necrosis factors (TNF).[24, 32, 33] Each cytokine has multiple activities and acts locally or systemically with overlapping functions.[34] The interactions of hormones and/ or cytokines initially stimulate osteoblasts and stromal cells, which are elaborate factors to signal osteoclasts in the regulation of bone resorption. It can be summarized in Fig.2.

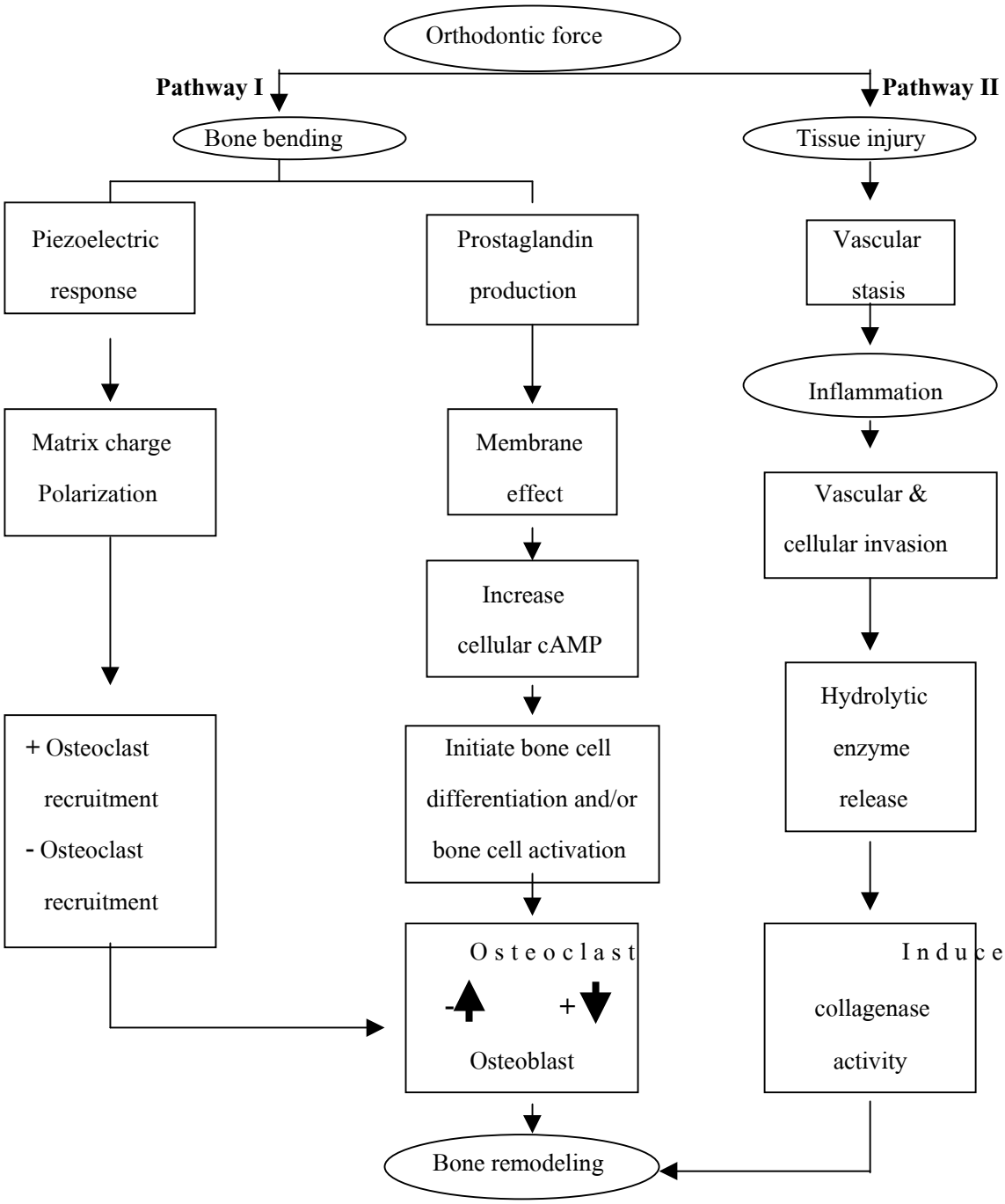

Fig.1: A flow chart of two biological pathways of orthodontic tooth movement. Pathway I shows a physiologic response and pathway II shows the production of a tissue inflammatory response. (26)

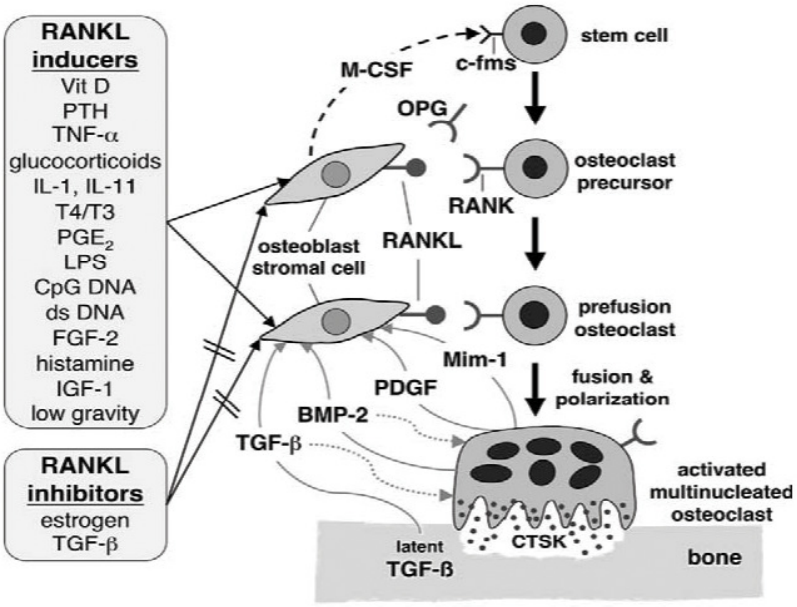

Fig.2: Diagram shows osteoclast differentiation, survival and activation. When receptor activator of NFkB ligand (RANKL) binds to the cytoplasmic membrane receptor activator of NFkB (RANK), subsequently stimulates both osteoclast differentiation and activation. Osteoprotegerin (OPG) inhibits this process, thereby preventing the combination of RANKL and RANK. Therefore, many cytokines and hormones can induce or inhibit osteoclast differentiation.[12]

Prostaglandins (PGs)

Application of an orthodontic force induces a local inflammatory process via the activation of phospholipase A2 which then activates the production of arachidonic acid from membrane phospholipids. Arachidonic acid gives rise to eicosanoids which are synthesized via 2 pathways; the cyclooxygenase pathway (COX pathway) and the lipooxygenase pathway. Eicosanoids are divided into 2 major classes; prostaglandins (PGs) and leukotrienes (LTs). Cyclooxygenase activity results in the formation of PGs and thromboxanes while lipooxygenase activity results in the formation of LTs and hydroxyeicosatetraenoic acids (HETEs).[35] PGs have 6 subgroups: PGD, PGE, PGF, PGG, PGH and PGI but the final products are PGD, PGE, PGF and PGI.[36]

PGE has been reported as biochemical mediators of bone resorption induced by orthodontic tooth movement.[17, 18, 24] Orthodontic force applied to the tooth stimulates the localized cells to synthesize and secrete PGE which, in turn, stimulates osteoclastic bone resorption.[15] PGE is known as a potent stimulator of bone resorption[37] and its production is controlled in part by IL-1β.[38] Grieve *et al*. (1994) found that the level of PGE_2 in human gingival crevicular fluid (GCF) was highest at 24 hours after the mechanical force was applied and it decreased to the baseline within 7 days.[22] The results from this study were consistent with those done by Lee *et al*. (2004)[24].

Yamasaki *et al*. (1984) demonstrated that injection of PGE_1 into human gingival tissue during orthodontic treatment increased approximately 1.6 fold the rate of tooth movement.[16] Leiker *et al*. (1995) showed that single and multiple administrations of PGE_2 at lower concentration had no effect on tooth movement, however, the multiple injections of a higher concentration of PGE_2 led to increase root resorption in rat.[39] Saito *et al*. (1991) reported that mechanical stress and IL-1β increased the production of PGE and enhanced the rate of tooth movement.[40] IL-1 has been shown to increase cyclooxygenase production by fibroblasts, resulting in increasing PGE production.[41]

Interleukins

Interleukins are one of the proinflammatory cytokines released from many cell types such as fibroblasts, macrophages, osteoclasts, polymorphonuclear leukocytes (PMN), etc.[20] They are soluble proteins or glycoproteins and serve as chemical communicators from one cell to another. They combine with surface receptors on target cells that are linked to intracellular signal transduction and second messenger pathways.[36] They have 18 types in family.[42] IL-1, IL-6 and IL-8 are proinflammatory interleukins that have been identified in GCF during orthodontic tooth movement.[9, 10, 21]

1. Interleukin 1 (IL-1)

IL-1 is a key mediator in immune and acute phase inflammatory responses. There are two forms of IL-1 found in GCF: IL-1α and IL-1β. IL-1α acts as a membrane associated substance, whereas IL-1β is found free in the circulation. They have the same biological activities and bind to the same receptor on cell surfaces. Both induce bone resorption but IL-1β seems to be a more potent inducer.[36] IL-1 is one signal transduction pathway in the osteoclast signaling which directly stimulates osteoclast differentiation, survival and activation (Fig.3).[12, 43] In addition, IL-1 is an inducer of IL-2[20], IL-6[9, 20], IL-8[25], Granulocyte Colony-Stimulating Factor (G-CSF)[20], PGE[44] and TNF[9, 10] in many cell types. The level of IL-1β was induced and reached a maximum level in 3 days after orthodontic force application then declined thereafter in rat.[9, 40] Ngan *et al*. (1988) showed an increase of IL-1β in human gingival fibroblasts during orthodontic movement.[45] Davidovitch *et al*. (1988) demonstrated that both IL-1α and IL-1β were increased in the areas of PDL tension and compression during orthodontic treatment.[13] In addition, several studies showed that the level of IL-1β in GCF during orthodontic tooth movement was increased and highest at 24 hours after the application of force and then declined.[10, 22, 24] Iwasaki *et al*. (2001) found that the velocity of human tooth movement positively correlated with the concentration of IL-1β and the correlation was stronger on the pressure site than the tension site.[23] AI-Qawasmi *et al*. (2003) reported that homozygous IL-1β allele 1 persons who produce decreasingly IL-1β have 5.6 fold risk of external apical root

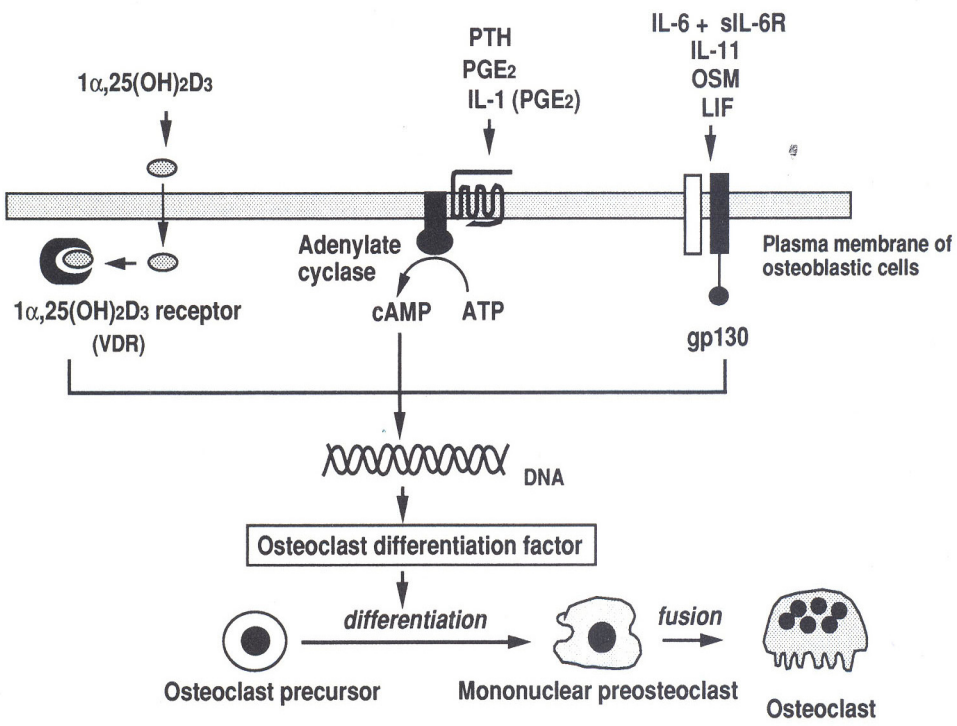

Fig.3: The signal transduction pathways induced by osteotropic hormones and cytokines in osteoclast differentiation. IL-1 is one signal transduction pathway in the osteoclast signaling which involved in the process of differentiation of postmitotic osteoclast precursors into functional osteoclasts.[43]

resorption comparing to unaffected individuals. Because the slowing down of bone resorption might result in prolong stress concentrated in the root, leading to root resorption.[46]

However, IL-1 has biological side effects. It can induce synthesis of enzymes that generate prostaglandins which may in turn induce fever. Shapira *et al*. (2003) reported that IL-1β level increased in GCF of erupting primary teeth which is correlating to clinical manifestations as fever, diarrhea, increased crying and sleeping and eating disturbances that occur at this time.[47] There has been reported that systemic administration of intravenous IL-1 from 1-100 ng/kg has produced fever, sleepiness, anorexia, generalized myalgias, arthralgias, headache

and some gastrointestinal disturbances while hypotension has been observed at higher doses. Additionally, the subcutaneous route is associated with less side effects.[48]

2. Interleukin 6 (IL-6)

IL-6 is released by lymphocytes. It modulates immune responses in inflammation. IL-6 can stimulate osteoclast formation resulting in bone resorption and bone remodeling. Alhashimi *et al*. (2001) applied orthodontic force on the rat maxillary first molar to study the level of IL-6 at 3, 7 and 10 days after force application. They found that the IL-6 level, induced by IL-1β, reached the maximum level in three days after force application and then declined.[9] Uematsu *et al*. (1996) studied the level of IL-6 in human GCF after canine movement at 1, 24 and 168 hours. They found that the level of IL-6 was increased and highest at 24 hours after force application and then declined.[10] In addition, Ren *et al.* (2002) qualified IL-6 level in GCF during orthodontic tooth movement in juveniles and adults. They found that the level of IL-6 was significantly elevated only in juveniles after 24 hours of orthodontic force application, which agrees the finding that the initial tooth movement in juveniles is faster than in adults.[49]

3. Interleukin 8 (IL-8)

IL-8 is a potent proinflammatory cytokine that plays an important role in the recruitment and activation of neutrophils and lymphocytes during inflammation. It is produced and secreted by many cells such as fibroblasts, epithelial cells, endothelial cells and alveolar macrophages, in response to inflammation.[20] It has been suggested to act as a potential regulatory signal for cell recruitment during bone remodeling.[50] Bendre *et al*. (2003) showed that recombinant human IL-8 can stimulate human osteoclastogenesis both dependent and independent of RANKL. It stimulates both the differentiation of human osteoclast precursors and bone resorption.[14] In another study observed serum levels of multiple cytokines in patients with postmenopausal osteoporosis. The result showed that the level of IL-8 was elevated. The investigators suggested that IL-8 may play a role in the high bone turnover associated with postmenopausal osteoporosis.[51] Tsai *et al*. (1995) reported that the IL-8 level was higher in periodontitis than in the healthy gingiva.[52] Conversely, Chung *et al*. (1997) demonstrated the

inverse relationship between IL-8 activity and PMN recruitment in the human gingival crevice of periodontitis patient suggesting that IL-8 is not the only factor controlling the influx and metabolic activity of PMN in the gingival crevice.[21] In addition, the levels of IL-8 in GCF were found to be positively correlated with IL-1β in periodontitis patients receiving supportive periodontal therapy.[25] After application of orthodontic force, Tuncer *et al*. (2005) evaluated the level of IL-8 in human GCF at baseline, 0 hour, 24 hours, 6 days and 30 days. They found that the IL-8 levels were increased and reached the maximum level on day sixth at the tension sites while the highest levels at the pressure sites were observed in the first and the 24th hour.[32]

However, various noninfectious diseases are known to be associated with neutrophilia and/or neutrophil infiltration into organs such as rheumatoid arthritis, gouty arthritis, respiratory distress syndrome, fever and so on.[48] Shapira *et al*. (2003) reported that IL-8 level increased in GCF of erupting primary teeth which is correlating with gastrointestinal disturbances that occur at this time.[47]

Tumor Necrosis Factor-α

Tumor necrosis factor-α (TNF-α) is of particular importance since it has been shown to be an early modulator of bone resorption and is detectable in the human gingival sulcus. Lowney *et al*. (1995) showed that there was a greater than two fold increase in TNF-α after application of orthodontic force.[53] Uematsu *et al.* (1996) evaluated the level of TNF-α in human GCF after orthodontic force application at 1, 24 and 168 hours. They found that level of TNF-α was elevated and highest at 24 hours after the force application and then declined.[10] But TNF-α mRNA was not detected during orthodontic tooth movement.[9]

Acid and Alkaline Phosphatase

Monitoring the levels of acid and alkaline phosphatase in serum and tissues are a common mean to assess bone turnover in human. Elevation in acid phosphatase activities is associated with bone resorption, whereas higher alkaline phosphatase activities are associated with bone formation.[54, 55] Insoft *et al.* (1996) found that the levels of acid and alkaline

phosphatase were increased during orthodontic tooth movement, however, they did not show a relationship to the plaque index. These inferred that phosphatase activities associate with bone turn over in tooth movement and they may be a useful means for monitoring tissue response to orthodontic treatment.[19]

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is the most important mediator for angiogenesis. VEGF was found to be able to stimulate monocytes aggregation and acts as a macrophage colony stimulating factor in osteoclast induction.[56]

Kaku *et al.* (2001, 2003) showed that the local administration of recombinant human vascular endothelial growth factor (rhVEGF) increases the number of osteoclasts in mice, which in turn resulting in increasing the rate of orthodontic tooth movement.[57, 58] Furthermore, recently, they investigated the effect of anti-VEGF antibody on osteoclastic differentiation, the amount of tooth movement and the degree of tooth relapse in mice. They found that the anti-VEGF antibody reduced the number of osteoclasts, inhibited tooth movement and relapse. These results suggested that anti-VEGF antibody may be useful in maintained anchorage and retained dental alignment after orthodontic tooth movement.[59]

Materials used to generate orthodontic force

Samuels *et al.* (1998) suggested that ideal physiologic force for human canine movement is approximately 150 cN.[6] In contrast, Boester *et al.* (1974) suggested to use the force between 100-300 cN for canine retraction.[60] There are many varieties of orthodontic material, giving different magnitude and type of forces, for canine retraction such as nickel-titanium closed coil spring (NiTi closed coil spring), elastic c-chain, active ligature, conventional elastic, magnetic, screw, etc. The common used materials in orthodontic clinic at Prince of Songkla University are NiTi closed coil spring and elastic c-chain.

The nickel-titanium close coil springs are made from nickel-titanium alloys (NiTi) which have two unique properties: the superelasticity and the shape memory phenomenon. The advantages are constant force delivery in relation to time of use and activation.[6, 61] In contrast, Nightingale and Jones (2003) found that NiTi closed coil spring lost force 48% over a time period of 1-22 weeks.[8] And Angokar *et al*. (1992) showed that the total force loss of NiTi closed coil spring was in the range of 8% to 17% after 28 days activation.[62] However, there are many studies reported that the NiTi closed coil springs give the most rapid rate of space closure and they can be used very easily for space closure in extraction cases.[1, 5, 6] Where as, Dixon *et al*. (2002) showed that the mean rate of space closure for the NiTi closed coil spring was higher than elastic c-chain although it was not statistically significant difference.[7] Therefore, the NiTi closed coil springs are still used extensively in orthodontic clinic for space closure and distalization of canine.[6] However, NiTi closed coil spring is more expensive than elastic c-chain.

Elastic c-chains are unable to deliver a continuous force over a period of treatment. Bishara *et al.* (1970) found that elastic c-chains (Alastiks, Unitek Corp.) suffered a 74 % loss of force delivery capability after 24 hours of load.[2] They recommended that elastic c-chain, when placed, needed to extend twice their original length to compensate for the large force loss during the first day. The initial force for elastic c-chain should be 300-400 cN. The initial force level exceeded 400 cN will interrupt the periodontal blood flow resulting in undermining resorption rather than frontal resorption for tooth movement and pain. In other words, Lu *et al.* (1993) showed that decayed force of the elastic c-chains was approximately 67 % in the fourth week.[63] The elastic c-chain will be changed after four week interval of time, because there appears a very little force which remained in the chain after this period. However, the elastic c-chain provides a cheaper treatment option than using NiTi closed coil spring.

Objectives and specific aims

Objectives

1. To determine the biochemical effect between using NiTi closed coil spring and elastic c-chain for canine retraction.
2. To use as data base to determine the proper method and material.

Hypothesis

1. The levels of IL-1β and IL-8 found in GCF between before and after 24 hours, 1 month and 2 months of force application are equal.
2. The levels of IL-1β and IL-8 found in GCF after 24 hours, 1 month and 2 months of force application between using NiTi closed coil spring and elastic c-chain are equal.

Benefits

1. To understand the biochemical effect of using NiTi closed coil spring and elastic c-chain for canine retraction.
2. To compare the biochemical effect and the efficiency between using NiTi closed coil spring and elastic c-chain for canine retraction.