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A study of the influence of combined Glucosamine Sulfate and Chondroitin Sulfate systemic supplements on root resorption and tooth movement in rats

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Dedication

To my wonderful wife, partner and best friend, Naomi. Thank you very much for your patience, understanding and unconditional support. I could not have completed this work without you.

To my parents Maha and Yehia. Thank you for making it possible for me to be in Australia and for your continuing love and support.

To my daughter Hana. Thank you for brightening up the last six months of my course.

Declaration

Candidate Certificate

This is to certify that the candidate carried out the work in this thesis in the Department of Orthodontics, University of Sydney and it has not been submitted to any other University or Institution for a higher degree.

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Nour Eldin Tarraf

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List of abbreviations

COX	cyclooxygenase enzyme
COX-2	cyclooxygenase enzyme 2
CS	chondroitin sulfate
CS4	chondroitin-4-sulfate
CS6	chondroitin-6-sulfate
cN	centi Newton
CT	computed tomography
DS	dermatan sulfate
e.g	for example
F	force
GAG	glycosaminoglycan
GCF	gingival cervicular fluid
GS	glucosamine sulfate
IL-1	interleukin 1
kg	kilogram
MMP	Matrix metaloproteinase
mg	milligram
microCT	x-ray micro-computed tomography
ml	milliliter
mm	millimeter
NO	nitric oxide
NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis
OIIRR	orthodontically induced inflammatory root resorption
OPG	osteoprotegrin
PG	prostaglandin
PGE2	prostaglandin E 2
PDL	periodontal ligament
PTH	parathyroid hormone
ppm	parts per million
RANKL	receptor activator for nuclear factor Kappa ligand
RANK	receptor activator for nuclear factor Kappa
SySADOA	symptomatic slow-acting drugs for osteoarthritis
TRAP	tartarate resistant acid phosphatase
TEM	transmission electron microscopy
TNSALP	tissue non-specific alkaline phosphatase
3-D	three dimensional
2-D	two dimensional
Δt	duration of the force

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1 Introduction

Orthodontic tooth movement is a periodontal ligament and alveolar bone phenomenon that involves microscopic and macroscopic changes in the periodontal ligament, alveolar bone and dental pulp. Root resorption is recognized as an unavoidable side effect of orthodontic tooth movement and numerous studies have been conducted in order to identify potential risk factors and possible methods to reduce or prevent this unwanted and often devastating side effect while maintaining or even improving the rate of tooth movement.

This review highlights the relationship between orthodontic tooth movement and root resorption and the various biologic mechanisms involved. It also examines the occurrence of root resorption as a side effect of orthodontic movement and the various clinical and systemic factors that contribute to tooth movement and root resorption. Several pharmacological agents have been examined and discussed with their relation to potentiate or retard orthodontic movement and their potential influence on reducing or preventing root resorption. Unfortunately to date there has not been a readily available pharmacologic agent that can be used as an adjunct with orthodontic treatment to reduce the risk of root resorption without affecting orthodontic tooth movement or inhibiting it. Glucosamine sulfate (GS) and Chondroitin sulfate (CS) are readily available over the counter nutritional supplements recently introduced with success in the management of Osteoarthritis (OA). This is attributed to their connective tissue building, as well as anti-inflammatory properties and their ability to regulate and reduce tissue breakdown. GS

and CS, especially CS, are present in the connective tissues of the periodontium as well as in cartilage and play an integral part in the metabolism of these tissues. Since inflammation is an integral part of tooth movement and the root resorption mechanism and matrix degradation with bone/cementum resorption are intimately related, GS and CS may have a role to play in modifying these mechanisms. The effect of GS and CS administration on tooth movement and root resorption has not yet been studied. Due to the increasing number of adults using GS and CS and the rising number of adults seeking orthodontic treatment it is important to investigate their potential effects on tooth movement and root resorption.

2 Biology of tooth movement and root resorption

For over 200 years dentists and orthodontists have been able to purposely move teeth based on the principle that, when a sustained pressure is applied to the teeth they will move in direction of the applied force. As early as 1904 experiments by *Sandstedt* also those by *Oppeneheim* tried to explain the biologic basis of orthodontic tooth movement (Sandstedt, 1904 and Oppenheim, 1911 , 1912). Their experiments showed that orthodontic force application introduces a variety of remodeling changes in the periodontal ligament, investing alveolar bone, dental pulp and gingival tissues. It was evident that sustained mechanical stress to the PDL induced extensive changes in cell activities and populations. The sequence of changes was well studied and described later by *Reitan* and others on microscopic and ultra structural levels (Reitan, 1951, Reitan,

1957, Rygh, 1972a, Rygh, 1972b). Several chemical mediators, cytokines and inflammatory mediators have also been identified (Krishnan and Davidovitch, 2006b) and recent studies have also examined the role of genetic factors and the expression of various genetic markers and cell surface receptors in tooth movement (Iwasaki et al., 2001b, Kanzaki et al., 2006, Kanzaki et al., 2004).

2.1 Tooth movement

2.1.1 Theories of tooth movement

Several theories have attempted to explain the process of tooth movement, the classical theory being the pressure tension theory. This theory relates tooth movement, at least in part, to the metabolic changes in the PDL subsequent to orthodontic loading, which alters the blood flow to the area. An alternative hypothesis explains tooth movement by bone bending and biologic electricity produced by the orthodontic force and evoking the cellular response. Both theories are neither contradictory nor mutually exclusive and both explain different aspects of the complex biologic mechanism behind orthodontic tooth movement.

2.1.1.1 The pressure tension theory

The pressure tension theory is considered the classical theory of orthodontic tooth movement (Meikle, 2006). It was born based on the histologic observations made by *Sandstedt* in the early 20th century (Sandstedt). The observations led them to believe that the tooth moves through the bone by creating a pressure side and a tension side (Schwarz, 1932). This pressure and tension in the periodontal ligament introduces various degrees of compression of the blood vessels which leads to changes in the blood flow and thus the oxygen tension and the chemical environment in the tissues. These changes lead to cellular proliferation and differentiation, with bone resorption in the pressure side and bone apposition in the tensions side (Meikle, 2006).

Sandstedt identified two ways the bone resorption took place in the pressure area. The first is the so called “direct bone resorption” or frontal resorption and the second is “indirect or undermining resorption” (Schwarz, 1932). *Schwarz* related their occurrence to the difference in the Magnitude of the applied force (Schwarz, 1932). He believed that if the force was large enough to cause pressure that exceeded the capillary blood pressure (26 g/cm3) it would cause strangulation of the blood supply and tissue necrosis in the areas of pressure which would lead to hyalinization and undermining resorption while if the force did not exceed the capillary pressure it would result in frontal resorption (Schwarz, 1932).

2.1.1.1.1 Frontal Bone Resorption (direct bone resorption)

It is believed to take place when light continuous force is applied to the tooth. The force is enough to produce compression and alteration of the blood flow but not great enough to obstruct the blood supply completely. Within a few seconds of force of application the tooth moves in its socket due to the expression of fluids from the PDL. The oxygen tension begins to change and chemical mediators such as prostaglandins and cytokines are released. This in turn activates the second messenger mechanism. *Davidovitch and Shanfield* demonstrated that after 4-6 hours elevated levels of cAMP, a well known marker for cellular activity and differentiation, are detectable in the PDL indicating beginning of cellular differentiation (Davidovitch and Shanfield, 1975). Another second messenger system has been demonstrated by *Sandy* which is the phosphoinositide pathway (Sandy and Farndale, 1991). These chemical messengers stimulate the differentiation of osteoclasts from the local cell population as well as evoking an inflammatory response and recruiting cells from the circulation. Osteoclasts are believed to arrive at the compression zone in two waves; the first wave is from the local cell population and the second wave via the blood flow from the circulating monocytes (Thilander et al., 2005). These cells then begin bone resorption of the adjacent alveolar bone from within the PDL space and tooth movement takes place shortly thereafter. It is estimated that it takes 2 days for tooth movement to occur this way. Osteoblasts on the other hand are recruited locally from progenitor cells in the PDL and begin bone formation on the tension side although lagging somewhat behind (Thilander et al., 2005).

2.1.1.1.2 Undermining bone resorption

This is believed to take place with moderate to heavy forces (Schwarz, 1932). In this case the force is large enough to occlude the blood flow to the tissues at the pressure side. This in turn results in a process of sterile necrosis within the compressed PDL (Schwarz, 1932). The tissue thus loses its normal tissue architecture and staining characteristics forming an avascular cell free zone which resembles the appearance of hyaline tissue under the microscope and thus was termed the hyalinised zone (Meikle, 2006).

The hyalinised zone is cell free and thus bone resorption takes place by recruitment of osteoclasts from adjacent non-hyalinised areas and also by undermining resorption from adjacent bone marrow spaces. This results in a delay in tooth movement of 7-14 days. This delay is due to the delay in cell stimulation and differentiation within the marrow spaces and the thickness of bone that needs to be resorbed before the osteoclasts can reach the hyalinised zone (Thilander et al., 2005).

The hyalinised zone has been described by *Roberts* (Roberts, 2005). He described packing of collagen fibers with hydrolysis of randomly coiled collagen chains; cells loose their cytoplasm and develop pyknotic nuclei and fibroblasts tend to accumulate in the compressed area followed by macrophages. Macrophages then remove the degraded fibrous tissue and cellular remnants and new capillaries begin to form in surrounding areas. Finally osteoclasts are formed in the marrow spaces adjacent to the hyalinised zone

after about 20-30 hours. The removal of the hyalinised zone is closely related to root resorption as will be discussed in detail in the section on the mechanism of root resorption.

Reitan concluded that because these observed changes were those of degeneration and were related to the force per init area in the PDL, attempts should be made to minimize the areas of hyalinization by reducing the force levels (Reitan, 1957). It is however agreed upon that frontal bone resorption alone is very difficult to achieve even under the most controlled experimental conditions and that hyalinization in some areas is inevitable. In reality both processes take place concomitantly but efforts should be made to minimize hyalinization as much as possible (Thilander et al., 2005).

2.1.1.2 Bone bending and tooth movement

Baumrind (1969) felt that the pressure tension hypothesis does not completely explain tooth movement. He argued that the periodontal ligament reacts as a continuous hydrostatic system, supported by the basic law of physics -namely Pascal's law- any force applied to the PDL would be transmitted equally to all regions. Due to the presence of a continuous body of liquefied ground substance the fibers in the PDL would not modify the operation of this law. In his experiments on rats *Baumrind* observed that the first molar was displaced 10 times more than the average reduction in the PDL width, upon force application, indicating that the bones ability to deform in response to force

may be greater than that of the PDL. *Grimm* produced similar findings in humans (Grimm, 1972).

Superficially this seems to contradict the orthopaedic dogma that states “any mechanical compression stimulates bone formation and tension stimulates resorption” (Melsen, 1999). *Zengo et al* in experiments on dogs demonstrated alveolar bone bending with canine movement to be no different from the bending of long bones that stimulates bone modeling (Zengo et al., 1973). When the tooth is loaded the alveolar bone wall on the tension side bends so that the PDL side of it becomes concave and thus the molecules and cells on the surface are under compression while the outer side of the bone becomes convex thus the molecules and cells on the surface are under tension. Compression will then stimulate deposition on the inside of the socket and tension stimulates resorption of the outside. The same applies to the pressure side; the alveolar bone bends so that the PDL side of the wall becomes convex and therefore under tension which stimulates resorption while the outside becomes concave thus the cells and molecules are under compression and stimulate formation.

Frost supported by Wolfs’ law explained how the stimulus for bone remodeling needs to exceed the minimum effective strain (MES) in order to evoke the regional acceleratory phenomena (RAP) that leads to bone remodeling.

2.1.1.3 Pathways of orthodontic tooth movement:

Mostafa et al presented an integrated hypothetical model for tooth movement (Mostafa et al., 1983). The model explains tooth movement in light of bone bending, biologic electricity and the pressure tension theory with the role of chemical mediators. The model consists of 2 pathways that work in tandem.

2.1.1.3.1 Pathway I

In this pathway the orthodontic force creates vectors of pressure and tension leading to bone bending and the generation of biologic electricity. Directionality of tooth movement was related to the difference in electrical charges between the concave and the convex sides of the strained alveolar bone. The electron neutral and positive areas promote osteoclastic activity while electron negative areas induce osteoblastic activity.

2.1.1.3.2 Pathway II

This pathway relates orthodontic tooth movement to the inflammatory response in the PDL following force application. This is through increased vascular permeability and cellular infiltration where lymphocytes, monocytes and macrophages invade the tissues with elevation of levels of prostaglandins and increased levels of cAMP with increase in osteoclast activity.

2.1.2 Phases of tooth movement

Burstone plotted the rates of tooth movement against time and identified three phases of orthodontic tooth movement namely an initial phase, a lag phase and a post lag phase. In the initial phase there is rapid movement for a very short distance immediately after force application (Burstone, 1962). This can be attributed to the displacement of the tooth in the PDL space and to the bending of the alveolar bone. This phase is followed by a period of no movement and is the lag phase. The lack of movement has been attributed to the development of the hyalinised zone in the areas where the PDL is under compression. The lag phase can vary considerably and is considered directly related to the age of the subject, density of the alveolar bone and the extent of the PDL areas of hyalinization (Roberts, 2005). It can also vary with the species studied (Reitan and Kvam, 1971). Tooth movement will only occur after the cells have removed the hyalinised zone and the underlying bone through undermining resorption entering the post lag phase (Thilander et al., 2005).

Recently a slightly modified model has been put forward by *Pilon et al* (Pilon et al., 1996). Their studies on dogs divided the curve into 4 phases. The first phase is similar to the previous model followed by a lag phase. The only difference is that once the necrotic tissue is removed tooth movement accelerates into the 3rd phase and continues into the fourth phase where the rate of tooth movement reaches a maximum.

2.1.3 The role of signaling molecules and inflammatory mediators

Several studies have demonstrated that the early phase of orthodontic movement involves an acute inflammatory response (Krishnan and Davidovitch, 2006b). It is characterized by the periodontal vasodilatation and migration of leukocytes which produce various cytokines that interact directly or indirectly with the local population of para dental cells. The cytokines together with other systemic and local signaling molecules evoke the synthesis and secretion of numerous substances by their target cells including prostaglandins, growth factors and cytokines (Davidovitch et al., 1976, Davidovitch et al., 1988). These cells are the units that then facilitate tooth movement by remodeling the para dental tissues (Krishnan and Davidovitch, 2006b).

The acute inflammatory response subsides within one or two days and is then replaced by a more chronic inflammation that is more proliferative in nature. This involves fibroblasts, osteoblasts endothelial cells and alveolar bone marrow cells. Leukocytes continue to migrate to the area of the stressed periodontal tissues and modulate the remodeling process. This continues until the force is reactivated (Krishnan and Davidovitch, 2006b).

Prostaglandins have been demonstrated to play an important role in tooth movement. Experiments on tooth movement have shown that levels of Prostaglandins and interleukin-1 beta are elevated in the PDL subsequent to orthodontic loading (Grieve et

al., 1994, Saito et al., 1991). It is believed that cells in the PDL respond to mechanical deformation by the release of prostaglandins (Harell et al., 1977) and so the release of prostaglandins may be a primary rather than a secondary response to the orthodontic force. Prostaglandin E has the unique property of being able to influence both osteoblastic and osteoclastic activity making it particularly important in tooth movement (Krishnan and Davidovitch, 2006b). Other inflammatory mediators and cytokines such as nitric oxide (NO) and IL-1 have also been identified in the PDL and the gingival cervicular fluid GCF subsequent to orthodontic loading (Davidovitch et al., 1988, Grieve et al., 1994). Several medications act by modifying the release of inflammatory mediators especially prostaglandins and so it becomes evident that pharmacologic agents may modify the response to orthodontic forces (Krishnan and Davidovitch, 2006a).

2.1.4 Extracellular matrix (ECM) and ECM remodeling

The ECM of the PDL plays an important role in tooth movement. It is composed of mainly collagenous fibers embedded in a gel-like ground substance of proteoglycans and glycosaminoglycan GAGs. It is believed that remodeling of the ECM plays an important part in orthodontic tooth movement. Immunolocalization studies have suggested a change in the proteoglycan profile in the PDL with orthodontic loading with increased expression of chondroitin 6 sulfate (C6S) in areas of compressive loading.

Many enzymes have been implicated in the remodeling of the ECM. An important group of enzymes are matrix metalloproteinases MMPs which include numerous enzymes that

have been identified together with their inhibitors TIMP (tissue inhibitors for MMPs) to play an important role in ECM remodeling (Apajalahti et al., 2003). Modifying or manipulating the function of the enzymes and their inhibitors can modify tooth movement as has been demonstrated by *Holliday et al* who showed that increased TIMPs can inhibit tooth movement (Holliday et al., 2003).

2.2 Root resorption

2.2.1 Definition and classification

Root resorption is defined as the active removal of mineralized as well as a thin layer of non-mineralized cementum (Brudvik and Rygh, 1994a). As early as 1856 *Bates* described the resorption of permanent teeth but the term ‘resorption’ was first used by *Becks* and *Marshall* in 1932 who defined it as the ‘destruction of formed tooth structure’ (Becks and Marshall, 1932).

Both physiological and pathological loss of root structure can be defined as external root resorption. Physiological resorption mainly refers to the resorption of the roots of primary teeth prior to their shedding and the eruption of permanent teeth. Pathological root resorption usually occurs secondary to an insult or pathological phenomenon but has also been known to be idiopathic (Thilander et al., 2005).

Ottolengui was the first to relate root resorption to orthodontic treatment while *Ketcham* in 1927 was the first to provide radiographic evidence of root resorption with orthodontic treatment (Ketcham, 1929, Ketcham, 1927).

Numerous classifications have been published categorizing the different types of root resorption. Proffit classified root resorption in relation to orthodontic treatment into three categories, the first being "moderate generalized resorption" which can be considered an

unavoidable complication associated with comprehensive orthodontic treatment in which all teeth included in treatment showed at least a small degree of blunting of the apices and up to 1-2mm of root shortening (Proffit et al., 2006). The second category of "severe generalized resorption" of all teeth is considered rare and not related to orthodontic treatment. However the third category of "severe localized resorption" can be considered an iatrogenic form directly linked to orthodontic force (Proffit et al., 2006).

In terms of the current understanding of the biology of tooth movement and the occurrence of root resorption, inflammation appears to play a major role in both processes. Therefore *Brezniak and Wasserstein* found it appropriate to term root resorption that occurs with orthodontic force orthodontically induced inflammatory root resorption OIIRR (Brezniak and Wasserstein, 2002a). This will be the definition used throughout this text.

Brezniak and Wasserstein classified OIIRR into three categories according to the degree of severity (Brezniak and Wasserstein, 2002a):

- Cemental or surface resorption with remodeling: this is resorption that only affects the outer layers of cementum and is fully regenerated.

- Dentinal resorption with repair (deep resorption): this process involves the resorption of the cementum and the outer layers of dentin which are then repaired

by cementum. The final shape of the root following this type of resorption may differ from its original form.

- Circumferential apical root resorption: this type of resorption results in root shortening due to the full resorption of the hard tissue components of the root apex. Varying degrees of root shortening are possible. When the root loses mineralized tissue no regeneration is possible.

2.2.2 Structure of cementum

Cementum is defined as a specialized mineralized connective tissue that covers the root surface of all human teeth. One of the main functions of cementum is to provide functional tooth support by anchoring the principal fibers of the periodontal ligament to the root surface. Root surface cementum also plays an important adaptive and reparative function in maintaining the integrity of the root surface (Ten Cate, 1998). Orthodontic tooth movement results in damage of cementum and to the root surface which sometimes reaches into dentine while the reparative process is solely by cementum (Brudvik and Rygh, 1995a).

Cementum is a component of the tooth itself but functionally it belongs to the periodontium or the tooth attachment apparatus. Although it has been suggested that it is a “bone like” structure it may only resemble bone in its mineral content. Firstly, unlike

bone, it does not contain any blood vessels or nerves and secondly, although it continues to grow in thickness throughout life it does not undergo a continuous mineral turnover (Ten Cate, 1998).

There is more cementum formation apically than cervically. Cementum is relatively thin at the cemento-enamel junction (CEJ). It is about 20-50 microns thick at the CEJ while it increases in thickness towards the apex to reach approximately 150-200 microns at the apex (Ten Cate, 1998).

According to *Ten Cate* 50-60% by weight of cementum is mineralized hydroxyapatite crystals with small amounts of amorphous calcium phosphate. The rest is 25% organic constituents mainly composed of collagen type I as well as collagen type III and a variety of non-collagenous proteins and 15% water (Ten Cate, 1998).

Cells involved in cementum formation are mainly cementoblasts as well as cementocytes and fibroblasts which originate from the ectomesenchymal cell population of the dental follicle. Cementum formation begins with the onset of root formation guided by the epithelial root sheath of Hertwig (Ten Cate, 1998).

Cementum has been classified according to the time of its formation into primary and secondary cementum which are formed by two distinct cementoblast populations. It has also been classified according to the presence or absence of cells into cellular and acellular cementum. Primary cementum is acellular cementum attached to the root dentin

and covers it from the cervical margin to the root apex. This type of cementum is covered by cellular secondary cementum. It is called cellular cementum since the cells that form it become trapped in lacunae within the cementum matrix in a similar manner to how osteocytes occupy lacunae in bone. Functionally acellular cementum anchors the periodontal ligament fibers while cellular cementum plays more of an adaptive role (Ten Cate, 1998). As will be discussed below, they also play a different role in root resorption repair.

2.2.3 Root resorption process

Early experimental studies by *Oppenheim* on dogs as well as those reported by *Sandstedt* described the occurrence of root resorption subsequent to orthodontic loading and tooth movement (Oppenheim, 1911 , 1912, Sandstedt, 1904). Schwarz theorized that if forces applied to the PDL exceeded the capillary pressure this would result in obstruction of blood supply and tissue necrosis (Schwarz, 1932). These areas of necrosis and tissue damage would then provoke resorption. A number of Scandinavian researchers who examined the biology of orthodontic tooth movement also described the mechanism by which root resorption takes place. *Reitan* described the occurrence of the so called "hyalanized zone" even with forces as low as 30g and that root resorption will occur at and around those areas of tissue damage (Reitan, 1951). These findings were also similar to those of *Kvam and Rygh* who found that root resorption was a side effect of the cellular activity associated with the removal of the necrotic tissue of the hyalinized area (Kvam, 1972b, Kvam, 1972a, Rygh, 1972a, Rygh, 1972b). Using scanning electron

microscopy (SEM) *Kvam* demonstrated root resorption lacunae extending into dentin but in areas where the hyalanized zone remained no resorption could be observed (Kvam, 1972b, Kvam, 1972a).

The cellular mechanism by which the root resorption takes place was well described in series of rat experiments by *Brudvik and Rygh* in the 1990s (Brudvik and Rygh, 1991, Brudvik and Rygh, 1993b, Brudvik and Rygh, 1993a, Brudvik and Rygh, 1994a, Brudvik and Rygh, 1994b, Brudvik and Rygh, 1995a, Brudvik and Rygh, 1995b). Those studies confirmed that the orthodontically induced inflammatory root resorption (OIIRR) is part of the process of eliminating the hyalanized zone. They found that initially mono-nucleated macrophage like cells which are negative for TRAP stain (tartarate resistant acid phosphatase) and have no ruffled border, begin the resorption process (Brudvik and Rygh, 1993b, Brudvik and Rygh, 1993a). Root resorption at the hyalanized zone occurs initially at the periphery around the necrotic area and is followed several days later by resorption of the root surface situated beneath the main part of the hyalanized zone (Brudvik and Rygh, 1993b, Brudvik and Rygh, 1993a). In the later stage of the resorption and beneath the hyalanized zone both multinucleated, TRAP positive cells without a ruffled border as well as mono-nucleated TRAP negative macrophage like cells were demonstrated to take part in the removal of the necrotic tissue and the root resorption (Brudvik and Rygh, 1994a, Brudvik and Rygh, 1994b). The multinucleated, TRAP positive cells without a ruffled border are considered to be osteoclasts or odontoclasts that did not come to full expression and with the introduction of a new mechanical

stimulus they become fully expressed in a matter of hours (Brudvik and Rygh, 1994a, Brudvik and Rygh, 1994b).

It has been suggested that the process of removing the hyalinized zone may damage the cementoblast layer on the outer surface of the root thus exposing the mineralized underlying cementum or it may be that the compression resulting from the orthodontic loading may damage the outer layer of cementum so that it also requires removal (Brezniak and Wasserstein, 2002a). It should be noted that removal of the root surface under the hyalinized zone begins only after the repair process has already begun at its periphery (Brudvik and Rygh, 1995b). The resorption process then continues until there is no more hyalinised tissue or until the force level is decreased. It is believed that the resorption expands the root surface and thus indirectly decompresses the root (Brezniak and Wasserstein, 2002a).

2.2.4 Root resistance to resorption and protective role of cementum

Several authors seem to suggest that the roots' outer surface layer in form of cementoblasts and cementoid or precementum plays a protective role against root resorption (Brezniak and Wasserstein, 2002a, Brudvik and Rygh, 1995a, Emslie, 1978).

The precise mechanism by which resorption is inhibited is not well understood. However there are several theories that attempt to explain this.

Firstly uncalcified mineral tissue such as osteoid (Chambers et al., 1984), predentin (Stenvick and Mjor, 1970) and cementoid (Gold and Hasselgren, 1992) have been found to be more resistant to resorption when exposed to orthodontic force. *Jones and Boyd* also suggest that Sharpey's fibers, precementum, predentine and elements of the organic matrix may still have a role in resistance to resorption (Jones and Boyd, 1988). These views have been opposed by *Andreasen* (1988).

More recent studies have also demonstrated that the process of bone resorption is regulated by the osteoblasts themselves. Recently an osteoclast activating factor RANKL (receptor activator for nuclear factor Kappa ligand) and its receptor RANK have been described using molecular techniques. RANKL and its receptor RANK expressed on osteoclasts and its precursor turned out to be determinants of osteoclast differentiation and function (Lacey et al., 1998, Yasuda et al., 1998). Another cytokine called osteoprotegrin (OPG) which is produced by osteoblasts and fibroblasts was found to inhibit osteoclastic function by competing with RANKL for its membrane receptor RANK (Simonet et al., 1997). Evidence is emerging that RANKL and OPG produced by periodontal ligament fibroblasts and osteoblasts play an important role in regulating tissue turnover and bone resorption during orthodontic tooth movement (Nishijima et al., 2006, Yamaguchi et al., 2006). Some evidence suggests that a similar role may take place by cementoblasts in root resorption (Yamaguchi et al., 2006) but evidence is not yet sufficient (Low et al., 2005).

2.2.5 Root resorption repair

Several studies have shown by light microscopy (Reitan, 1974) and SEM (Harry and Sims, 1982, Barber and Sims, 1981) that after the termination of orthodontic force repair of the resorption lacunae takes place by the deposition of new cementum. *Brudvic and Rygh* examined the ultra-structural changes that take place with root resorption repair using transmission electron microscopy (TEM) on the rat model (Brudvik and Rygh, 1995b, Brudvik and Rygh, 1995a). Their results indicated that the process of repair can take place even in the presence of a light force. The process is associated by the invasion of fibroblast like cells from the circumference into the root resorption site (Brudvik and Rygh, 1995b). After ten days of loading it was already evident that the repair process and the formation of new cementum was occuring at the periphery while odontoclast like cells were resorbing the tissue at the centre of the lesion. After the termination of the force it was evident that new cementum formation was taking place in similar fashion to what occurs during tooth development. By 21 days after force termination new mineralized cementum could be seen at the depth of the resorption lacunae, with the structures of the PDL reattaching and appearing very similar to the control samples (Brudvik and Rygh, 1995a). *Owman-Moll and Kurol* on human premolars found that the repair process starts initially by the formation of a layer of acellular cementum and is quickly covered by cellular cementum which then is major repair tissue (Owman-Moll and Kurol, 1998a)(Owman-Moll et al., 1995b). The repair takes place by migration of cementoblasts over the resorbed surface (Jones and Boyd, 1988).

2.2.6 Incidence of root resorption

Root resorption is not a phenomenon unique to orthodontic treatment but has also been reported among untreated individuals. *Henry and Weinmann* studied permanent teeth from 261 persons and found evidence of root resorption in 90% of the teeth (Henry and Weinmann, 1951). The majority of the resorption areas were found to be related to the apical third of the root while some resorption was found in the middle third and very little in the gingival third. Some level of root resorption may be considered a normal physiologic process related to continuous bone and tissue remodeling or physiologic tooth migration (Vlaskalic et al., 1998).

In spite of the fact root resorption is a normally occurring phenomenon it is a particular problem in orthodontics due to the increased amount of resorption that occurs with treatment.

Among orthodontically treated individuals clinical and experimental research shows that some amount of root resorption (OIIRR) is an unavoidable side effect of orthodontic treatment (Proffit et al., 2006). The maxillary incisors seem to be more at risk, particularly the lateral incisors, but more or less most teeth included in the appliances will be affected (Ahlgren, 1993, Kaley and Phillips, 1991, Linge and Linge, 1983, Linge and Linge, 1991, Mirabella and Artun, 1995b, Mirabella and Artun, 1995a, Remington et al., 1989, Sameshima and Sinclair, 2001a, Smale et al., 2005). The mean amount of resorption in the samples mentioned above was very similar and stayed at less than

1.5mm in the studied groups. Although this may indicate that the amount of resorption may be clinically insignificant for the average orthodontic patient, when examining the data further it seems that there is great individual variation.

Examples of studies showing such large variations include *Linge and Linge* (1983), who in a sample of 719 consecutively treated patients found that the mean resorption for the four maxillary incisors was 0.7mm but 2 % of adolescent patients showed at least one tooth that resorbed more than 5mm during the treatment period. They also reported that the mean for the most affected tooth was 1.34mm in their sample. In another study *Mirabella and Artun* concluded that OIIRR is not more prevalent among adult orthodontic patients after examining a sample of 343 adult patients with intra oral radiographs before and after orthodontic treatment (Mirabella and Artun, 1995a). In their group the mean resorption was 0.93 mm with the most affected tooth mean being 2.39 mm. But it is worth mentioning that 40% of the adults had one or more teeth that suffered 2.5mm of resorption or greater. Most of the above studies were retrospective in nature. The resorption is known to be evident quite early in treatment, in a multi centre prospective study *Smale et al* (Smale et al., 2005) demonstrated that root resorption incidence is evident early in treatment in the leveling and alignment phase and is a good indicator of the severity of resorption to be expected at the end of treatment. They demonstrated that about 4.1% of patients studied suffered an average of 1.5 mm resorption or more in the maxillary four incisors. They also showed that 15% of patients had 1 or more maxillary incisors that suffered 2 mm or greater resorption after 3-9 months of treatment. These findings are in agreement with those of an earlier study by

Levander and Malmgren who found that root resorption at the end of treatment was correlated to severity of the resorption at 6-9 months from initiation of treatment (Levander and Malmgren, 1988).

From the above it is evident that some degree of root resorption is unavoidable with orthodontic treatment and although in many patients it may be clinically insignificant, some patients seem to suffer more resorption then others. The following section will discuss the risk factors that predispose to OIIRR.

3 Factors that affect tooth movement and the risk of root resorption

Several factors can affect tooth movement and the occurrence OIIRR. Some factors can be related to the treatment and orthodontic mechanotherapy while others can be related to the local tissue environment and the teeth involved. Other factors are related to the host physiology and can be affected by medications and changes in the physiology of the body. The following section will discuss the effect of treatment related factors followed by local environmental factors and then the effect of host physiology and systemic factors and finally the effect of medication on tooth movement and root resorption.

3.1 Treatment related factors

3.1.1 Proximity to the cortical plate

A case control study *Kaley and Phillips* found that in patients where the maxillary incisor roots had approximated the cortical plate, the risk of severe resorption increased by twenty fold(Kaley and Phillips, 1991). These findings are also similar to those of (Horiuchi et al., 1998) who found that maxillary incisor retraction by forcing them into the cortical plates is a significant factor in severe root resorption. Patients at risk are those with skeletal problems that are treated by dental camouflage with extractions (Proffit et al., 2006).

3.1.2 Type of appliance and different orthodontic mechanics

Several studies have attempted to examine the effects of using different types of orthodontic appliances with regards to OIIRR. Studies have compared removable to fixed appliances (Linge and Linge, 1983), Begg technique to the edge wise technique (Parker and Harris, 1998, L'Abee and Sanderink, 1985, Goldson and Henrikson, 1975), lingual verusus labial appliances and self ligating versus conventional ligation (Blake et al., 1995).

With regards to removable versus fixed appliances it was shown that removable appliances produced less resorption than fixed appliances (Linge and Linge, 1983). This

finding is very logical considering that removable appliances are limited in the amount and type of tooth movement they can achieve they are also limited in their range of activation. More importantly they do not deliver a continuous force since they are likely to be removed during the day which allows tissue recovery and more repair than with fixed appliances.

It is unlikely that the type of appliance itself is a decisive factor in the risk of root resorption as it is the force transmitted to the tooth that really matters. It comes as no surprise that most of these studies have demonstrated no statistical difference between the appliances with regards to root resorption but instead have related the extent of resorption to the duration of treatment and the amount of tooth movement achieved (Blake et al., 1995, Parker and Harris, 1998).

3.1.3 Maxillary expansion

Maxillary expansion is of particular interest because the forces involved are considerably greater than those used with conventional appliances. The tooth roots act as anchorage for orthopaedic expansion of the maxilla and thus are compressed against the thin buccal cortical plate. *Isaacson* found that a single activation of jackscrew appliances produces forces in the 3 to 10 pound range, while multiple daily activations can result in cumulative loads of 20 pounds or more (Isaacson and Murphy, 1964, Isaacson and Ingram, 1964). After the short period of expansion the appliances are left in place to retain the orthopaedic expansion with the tissue recoil loading the teeth in reverse. *Reitan*

stated that even though appliances may be passively holding the teeth in position, if this position is unstable then the tissue recoil will maintain the pressure on the teeth and allow the root resorption process to continue (Reitan, 1974). This was also shown by the findings of *Barber and Sims* (1981) who found that the root resorption process was still active after 9 months of retention with the appliance. It has also been demonstrated that not only do the loaded teeth show resorption with rapid maxillary expansion but also unloaded teeth. *Vardimon et al* showed that central incisors may suffer considerable resorption in cats after maxillary expansion (Vardimon et al., 2005).

3.1.4 Force magnitude

The effect of force magnitude on the efficiency and rate of tooth movement has been long debated in the orthodontic literature. Based on the classical views of the pressure tension theory it is believed that if orthodontic forces are used that exceed the optimal level, then ischemia develops in the PDL at the pressure site leading to tissue damage and the development of the so called “hyalinised zone”. This will then halt tooth movement until the damaged tissue is removed; bone resorption takes place with undermining resorption. If optimal forces are used then the result is ‘direct’ or frontal resorption with minimal or no tissue necrosis. A recent systematic review by *Ren et al* attempted to identify the optimum force level for efficient tooth movement (Ren et al., 2003a). The authors concluded that examination of both animal and human research failed to identify a force magnitude that is considered optimal. This is firstly due to the wide variation in animal species used as well as the variation in modes of loading, direction of tooth movement

and duration of the experiments. The authors also found that most studies did not report about the relationship between magnitude of the force and the rate of tooth movement. With regards to human studies similar problems were faced with small sample sizes being a particular problem making individual variations too great to make any definite conclusions.

It is also worth mentioning that human studies that have used similar force magnitudes as well as different magnitudes found that individual response is very variable for the same force application and pointed towards the genetic makeup and individual genotype making tooth movement more efficient in certain individuals than in others (Iwasaki et al., 2008). Furthermore two recent reports by *Von Bohl et al* (Von Bohl et al., 2004a, Von Bohl et al., 2004b) questioned the validity of the models that describe the relationship between the magnitude of the orthodontic force and the rate of tooth movement. According to their findings the rate limiting factor for orthodontic movement is the degree of hyalinization that occurs following the application of the force and the removal of this necrotic tissue. This is largely dependent on the strain distribution in the periodontium and the peculiarities of the PDL and bone morphology.

Root resorption has been identified to take place at the “hyalinized zone” in relation to tissue damage with those areas of the root being “marked” by the damage and resorption taking place. For this reason *Reitan* suggested using light forces in orthodontic treatment to allow the stimulation of a cellular response while minimizing the side effects in the form of root resorption (Reitan, 1964). *Vardimon* explained the determinants of root

resorption in response to loading with the magnitude of force being a significant determinant in combination with the duration of force application (Vardimon et al., 1991).

Some controversy in the literature exists as to the effect of force magnitude on OIIRR. Several animal studies have demonstrated a relation between the magnitude of the applied force and the amount of root resorption with heavier forces causing more resorption than light forces.

King and Fischlschweiger (1982) used a rat model and reported little cemental cratering with light forces of 40g compared to substantial cratering with 300g after two weeks of loading. This was also in agreement with *Dellinger* (1967) whose study on monkeys found that 10g and 50g of intrusive forces caused moderate amounts of root resorption while increased resorption was seen with 100g and severe resorption with 300g. *Vardimon et al* loaded maxillary premolars in monkeys with a buccaly directed force and found that magnitude of force is a major determinant of root resorption in short time periods but the duration of force application plays a major role when longer durations are considered (Vardimon et al., 1991).

On the other hand in a well controlled study by *Maltha et al* on dogs the results were different (Maltha et al., 2004). The study used several orthodontic forces and two different loading regimes continuous and intermittent forces respectively. A specially designed appliance that produces bodily movement was used. They found that the force

magnitudes of 10, 25, 50, 100 and 200 cN did not show a significant difference in the amount of root resorption while duration and mode of loading showed significant differences. It should be noted that they used a controlled bodily movement while others have used uncontrolled tipping (King and Fischlschweiger, 1982) which means stress concentration in the PDL would be quite different (Thilander et al., 2005).

Human studies have also demonstrated opposing results. Several studies from the University of Sydney demonstrated an almost linear relation between force magnitude and amount of root resorption (Chan and Darendeliler, 2005, Harris et al., 2006, Srivicharnkul et al., 2005). *Chan and Darendeliler* used light buccaly directed force of 25 g and 225g for the light force and heavy force groups respectively (Chan and Darendeliler, 2005). They used a loading period of 28 days with TMA springs. Using volumetric analysis of the root resorption craters they demonstrated that the light force group had almost 3.5 fold the volume of resorption compared to the control group while the heavy force group showed 11.5 fold the resorption volume of the control group and 3.3 times the resorption of the light force group. In another study *Harris et al* using a similar experimental design but using intrusive forces a similar result was obtained (Harris et al., 2006). Light and heavy forces caused 2 and 4 fold the volume of resorption of the control group respectively. Those findings are also supported by the work of Harry and Sims (1982) on human premolar intrusion.

These results are in contrast to what *Owman-Moll et al* (Owman-Moll et al., 1996b, Owman-Moll et al., 1996a) showed on a similar human model. *Owman-Moll et al* tested

the effect of doubled orthodontic force on root resorption (Owman-Moll et al., 1996a). They used a buccaly directed force of 50cN and 100cN respectively. Serial histological sections were taken to assess the root resorption. Surprisingly they found that resorptive lesions appeared more frequently in the light force group compared to heavy force group but there was no difference when the depth and extension of the craters were analyzed. In a further study by the same group (Owman-Moll et al., 1996b) a four fold increase in the orthodontic force, 50cN compared to 200 cN, did not seem to show a significant difference in resorption. These findings are also similar to those of *Reitan* (1974). Examining 72 human premolars after loading with various intrusive, extrusive and tipping forces ranging from 25-240g *Reitan* found that OIIRR was poorly correlated with the magnitude of force.

The disparity between the results of the studies can be due to various reasons. Firstly the definition of heavy and light forces is not very clear, what some authors describe as heavy force was less than half that described by others (Ren et al., 2003a). It is also not clear in the orthodontic literature as to what force is considered heavy and if there is a force limit that if crossed, tissue reaction would be significantly different (Ren et al., 2003a). Secondly the type of tooth movement and the designs of the appliances differ from one group to the other, while some tried to replicate tipping others tried to intrude and others where using bodily movement. It is well documented that different modes of loading create different distribution of stress in the PDL (Thilander et al., 2005) and thus the areas of stress concentration would be different which may affect the root resorption presenting. Thirdly forces used in animal models do not translate very well to human

subjects. It is difficult to assess what a 50 cN force in a rat models would resemble on a human model (Ren et al., 2004). Lastly the methods used for the measurement and the quantification of root resorption are vastly different making comparisons between the results of the studies difficult if not impossible. An example is the study by *Chan and Darendeliler* (2005) and that by *Owman-Moll et al* (1996b) although both studies copied the appliance design by *Lundgren et al* (1996) they used different methods to quantify the root resorption producing opposing results. This is also in addition to the great variation in loading times, wire materials and appliance reactivation regimes.

3.1.5 Duration of force application and amount of tooth movement

Clinical experience and experimental research suggest that a threshold of force duration of around 6 hours per day is required for successful tooth movement. Experiments by *Davidovitch and Shanfield* on cats showed that it takes around 3 hours of continuous force application in order for the biologic mechanisms controlling tooth movement to be initiated with elevation of cAMP levels in alveolar bone extracts (Davidovitch and Shanfield, 1975).

The duration of force application is believed by some authors to be a very important factor in governing the amount of root resorption in orthodontic treatment (Maltha et al., 2004). Longer duration of the applied force means more time for the cemento clastic activity while allowing little time for the repair process, of course this will also relate to

the type of force application whether it is continuous or intermittent (Maltha et al., 2004). In a number of radiographic retrospective studies several authors have reported that longer treatment periods can be a risk factor for increased resorption (Baumrind et al., 1996, Linge and Linge, 1991, Sameshima and Sinclair, 2001b, Sameshima and Sinclair, 2001a, Segal et al., 2004). On the other hand others have shown the treatment duration not to be a significant risk factor (Hendrix et al., 1994). It is difficult from retrospective studies to examine the effect of treatment duration as it is possibly the duration of active treatment where tooth roots are being moved that plays an important role not the overall treatment duration. Cases where the treatment time has been prolonged may have had periods when the appliances were in a passive state in one arch while more work needed to be done in the other arch. Prolonged treatment can also be due to missed appointments or frequent breakages and so it may be difficult to rely on the duration variable alone. To make the duration of treatment more pertinent some authors have correlated the amount of resorption with active periods of treatment for example *Linge and Linge* (1991) found a correlation between the amount of resorption in anterior teeth and the time in rectangular wires and the period where class II elastics were employed it may still be argued that rectangular wires are not necessarily active in all the patients and probably not to the same degree while increased periods of class II elastics could be due to the lack of compliance. A more representative approach was to relate the duration of treatment and the amount of apical displacement of the teeth which better represents the active treatment period. This approach was taken by *Baumrind et al*, they found a significant relationship between the amounts of apical root resorption of incisors with the amount of horizontal apical displacement as measured from lateral head films(Baumrind et al.,

1996). Surprisingly vertical apical displacement was not a significant factor. These findings were also confirmed by *Sameshima and Sinclair* (2001a) who also found that extraction cases showed more resorption than non extraction cases which also was consistent with the duration of treatment being longer with more apical displacement. Furthermore a recent systematic review by *Segal et al* on the effect of treatment related factors and root resorption found that the greatest predictors for the amount of root resorption are the amount of apical movement of the tooth as well as the duration of active treatment (Segal et al., 2004).

Vardimon developed a formula that explains the interaction between the force magnitude and duration on OIIRR (Vardimon et al., 1991). His findings indicate that the root resorption is governed by the impulse which is force (F) multiplied by the duration the force is acting Δt ($F \times \Delta t$) and the critical barrier of periodontal ligament as the primary determinants.

In order to examine the role of force duration a more prospective and controlled experimental design needs to be carried out. *Owman-Moll* (1995) conducted a large study on 144 human premolars with a buccally applied force. Among the variables tested was the effect of force duration. Although the authors found that the force decayed, an effort was made to regularly reactivate the appliances to maintain continuous loading. The authors found that at 3 weeks 93% of the teeth showed resorption sometimes half way through to the pulp. The amount of resorption increased with time. *Kurol et al.* also showed similar findings (Kurol et al., 1996). These results are similar to what *Maltha et*

al. (2004) reported; the study was a well controlled study on dogs in which different magnitudes of force, loading regimes and loading durations were tested. After 7 days root resorption could already be seen on some root surfaces and at 2 weeks 16% of root surfaces were showing resorption at the pressure side. By 14-17 weeks it was obvious that resorption was progressing with time with 94% of root surfaces at the pressure side showing root resorption. Root resorption was quite extensive ranging from 6-77% of the root surface.

It is important to mention that from the above it seems that force duration may play a key role in OIIRR but the discussion of duration is not complete until the loading regime is also discussed. Whether the force applied over a certain period of time was a continuous or an intermittent or decaying force can play an even bigger role in root resorption. This leads to the next point in this review which is the role of continuous versus intermittent forces.

3.1.6 Continuous versus intermittent force

Studies have demonstrated that continuous forces are more efficient in tooth movement than intermittent or decaying forces (Darendeliler et al., 1997, Krishnan and Davidovitch, 2006b). This becomes evident from the fact that most contemporary orthodontic appliances employ light continuous forces as part of their mechanotherapy (Krishnan and Davidovitch, 2006b).

However, with regards to limiting root resorption using intermittent forces may be of an advantage. It is well documented that root resorption is closely related to the “hyalanized zone” and areas of tissue and blood vessel compression and damage in the compressed areas of the PDL (Reitan, 1972). Intermittent forces probably prevent the formation of such “hyalanized zones” or allow reorganization of the tissue in the compressed areas and restoration of blood flow to the tissues in those periods when the force is inactivated (Reitan, 1957). This may lead to less aggressive resorption. Further more, an intermittent force will allow the repair process to be initiated and for repair to occur in the damaged areas in between periods of activation (Rygh, 1977). A continuous force will not allow the repair of the damaged cells and blood vessels and thus results in more extensive and aggressive resorption. Several animal and human studies have examined the effect of continuous and intermittent forces on root resorption. In a rat study *Kameyama and coworkers* showed that periods of inactivation of 4 and 9 hours daily reduced the amount of root resorption significantly while 1 hour inactivation did not reduce root resorption significantly as compared to no inactivation (Kameyama et al., 2003). It should be noted that inactivation also significantly reduced tooth movement as well. Similar results were also demonstrated by *Maltha et al* who found that using an intermittent force with a period of daily inactivation of 8 hours produced 40-70% less root resorption (Maltha et al., 2004). The clinical applicability of such a loading protocol is very difficult and practically impossible.

Several clinical studies have tried to replicate more life like situations (Acar et al., 1999, Owman-Moll et al., 1995a, Weiland, 2003). *Acar et al* employed elastics applied continuously on one side and only 12 hours a day on the other side to introduce a tipping movement (Acar et al., 1999). Although the use of elastics is less than ideal for continuous forces and patient compliance was not assessed (King, 1999) the study showed that intermittent force produced less resorption than continuous force. *Weiland* (2003) on the other hand used a better design that may resemble clinical applications. They used a super-elastic NiTi wire to apply a buccally directed force on the second premolar on one side of the arch while they used a stainless steel wire to do the same thing on the other side. Super-elastic NiTi wires are known to produce a constant force over an extended portion of their deactivation range compared to stainless steel which exhibit rapidly decaying force during activation (Kusy, 2002). Their results showed that with continuous force the volume and the area of the resorption craters was 140% greater than with the dissipating force. One study did not however corroborate these findings. *Owman-Moll et al* investigated the effect of continuous and a continuous interrupted force on human premolars (Owman-Moll et al., 1995a). They used a 50cN spring on one side which was reactivated weekly to represent continuous force while on the other side the spring was only activated once and then left uncontrolled for 3 weeks and then made passive for one week for tissue recovery. The study showed that continuous force produced more tooth movement while there was no difference in root resorption between the loading regimes. This study was criticized for several reasons and their results can be considered quite misleading (King, 1995). The sample size may have been too small and individual variations reported were significant. The continuous force used was not as

such considering the reactivation was weekly and the authors reported a 22% decay of the force over the week (King, 1995).

From the above it seems that the majority of the literature indicates that continuous forces are more efficient at producing tooth movement but cause more root resorption than intermittent or dissipating forces.

3.2 Local factors

3.2.1 Dentition stage

The evidence in this area seems to be some what contradictory. *Linge and Linge* (1983) concluded that incisors that are orthodontically treated before the age of eleven show less resorption than if treated after that age, they related this to the apical maturation. Teeth with open apex seemed to show less resorption. On the other hand *Hendrix et al* showed age was not a significant factor but teeth with incomplete root formation at the beginning of treatment continued to show root lengthening during active treatment however they did not reach their normal tooth length (Hendrix et al., 1994).

3.2.2 Type of malocclusion

There is no malocclusion that is immune to root resorption but several studies have found correlation between the amount of root movement and the amount of OIIRR (Segal et al., 2004). This explains why increased over jet and extraction cases have demonstrated more severe resorptions (Linge and Linge, 1991, Harris et al., 2001, Harris and Baker, 1990, Brin et al., 2003).

3.2.3 Endodontic treatment

There has always been a concern with regards to orthodontically moving endodontically treated teeth. It was initially thought that these teeth may not respond as readily to orthodontic force or they may be more susceptible to root resorption. Since it is the response of the PDL, not the pulp, that is the key element in orthodontic tooth movement, moving endodontically treated teeth should be perfectly feasible (Proffit et al., 2006). Both animal (Mah et al., 1996) and human studies (Wickwire et al., 1974) show that endodontically treated teeth can be moved orthodontically as readily as vital teeth.

There is some evidence in the literature that suggests that endodontic treatment may offer some protective function for the roots reducing the risk of OIIRR (Hamilton and Gutmann, 1999). Although an earlier study by *Wickwire et al*. (1974) suggested that endodontically treated teeth are more susceptible to resorption than vital teeth, most recent studies suggest that is not the case. *Spurrier et al* studied 45 orthodontic patients with one or more endodontically treated teeth before orthodontic treatment and who exhibited signs of apical root resorption after treatment (Spurrier et al., 1990). They found that the vital contralateral teeth, which served as controls, exhibited statistically, though not clinically, a significantly greater amount of root resorption (0.77mm) than those that had been treated endodontically, this was also in agreement with *Mirabella and Artun* (Mirabella and Artun, 1995a). On the other hand a more recent study (Esteves et al., 2007) indicated no difference in the amount of apical resorption between the two groups.

An exception appears to be those teeth that have a history of trauma, especially an intrusive type of trauma. These stand a 50% chance of suffering moderate to severe resorption during orthodontic treatment (Chaushu et al., 2004).

3.2.4 History of trauma

Traumatized teeth in general and especially those that suffered an intrusive trauma seem to suffer more root resorption without orthodontic treatment. It has also been suggested that those teeth would be at a greater risk of resorption with orthodontic treatment (Andreasen, 1988).

It is believed that trauma may damage the protective outer layer of cementum and thus exposing the underlying mineralized cementum to resorption which is aggravated by the presence of inflammation (Andreasen, 1988).

Several studies which were mostly retrospective have examined this risk factor with some conflicting results. In two retrospective studies *Linge and Linge* have identified a history of trauma to be a risk factor for increased OIIRR (Linge and Linge, 1991, Linge and Linge, 1983). This was also in agreement with *Brin et al.* (1991) who found that teeth with a history of trauma showed more resorption with removable appliances than did those without a history of trauma. On the other hand others have found that not to be the case. *Malmgren et al* found root resorption in cases treated with fixed appliances to be no

different in teeth with history of trauma than those without although they did indicate that teeth with history of trauma that are already showing signs of root resorption may be at greater risk of resorption during treatment (Malmgren et al., 1982). *Brin et al* also confirmed those findings (Brin et al., 2003).

It is difficult to reach a definite conclusion with regards to the effect of trauma on the susceptibility to OIIRR from the above mentioned studies. This is due to a number of reasons. Firstly in retrospective studies examining patient records it may not be clear what kind of trauma is in question. If a patient reports a history of trauma it is difficult to assess the severity of such trauma and also the type of trauma. Intrusive trauma is reported to be one of the most serious affecting the PDL and seems to be more associated with root resorption and ankylosis (Andreasen et al., 2006). *Chaushu et al* have also reported that endodontically treated teeth with history of intrusive trauma stand a 50% chance of developing severe root resorption with orthodontic treatment (Chaushu et al., 2004). Without accurate history of the kind of trauma sustained it is difficult to compare the results of those studies. Secondly it is not clear whether the trauma sustained may have caused pulp necrosis or obliteration which may have passed undetected prior to orthodontic treatment and then later contributed to the inflammation and thus exacerbated the OIIRR.

3.2.5 Root morphology and dental anomalies

Several studies have reproted that root morphology may be a risk factor for OIIRR (Levander and Malmgren, 1988, Sameshima and Sinclair, 2004). They found that blunt and pipette shaped roots had a significantly higher chance of developing root resorption, with pipette shaped roots being at the highest risk. Several other studies have indicated that abnormal root morphology may predispose teeth to increased OIIRR (Brezniak and Wasserstein, 2002b). *Sameshima et al* found that the lateral incisors were the most severely affected teeth in their sample and that they were also the teeth with the greatest percentages of abnormal root shapes (Sameshima and Sinclair, 2001a). On the other hand *Kook et al* found no difference in resorption between peg shaped laterals and normal laterals but found small lateral incisors to be more susceptible to resorption (Kook et al., 2003). *Sameshima and co-workers* also looked closer at their sample of 868 patients and identified the ones that showed the most severe resorption and found that abnormal root morphology was a major factor (Sameshima and Sinclair, 2004). This was also in agreement with previous reports by Mirabella and Artun (1995a) as well as Brin et al (2003).

Furthermore there has also been a link found between the presence of dental anomalies and the development of severe OIIRR. *Thongudomporn and Freer* studying the records of 111 patients, found that patients with any one dental anomaly were at significantly greater risk of developing OIIRR than those who have no anomalies (Thongudomporn

and Freer, 1998). Dental invaginations, dilacerations and pipette shaped roots were among the described anomalies. On the other hand *Mavragani et al* found no relation between mild, moderate or severe dental invaginations and root resorption (Mavragani et al., 2006). They did however point out that teeth with dental invaginations were more likely to also have abnormal root morphology which could be a reason for increased OIIRR.

Patients with multiple agenesis were also identified as high risk patients. *Levander et al* examined patients with multiple aplasia of teeth and found those with four or more missing teeth seemed to exhibit more resorption than those who are missing three teeth or less (Levander et al., 1998). However these results should be regarded with some caution since these patients may have also been subject to more extensive orthodontic treatment with more tooth movement than the average patient and most likely longer duration. The authors pointed out that in the same sample there was a significant relation between the severity of resorption and the duration of treatment.

3.2.6 Habits

Historically it has been suggested that oral habits may be associated with the development of root resorption (Newman, 1975).

In two publications *Odenrick and Brattstrom* (Odenrick and Brattstrom, 1985, Odenrick and Brattstrom, 1983) linked severe nail biting habits during orthodontic treatment with

increased level of root resorption. It is hypothesized that the jiggling action from the habit may be behind it. It is however difficult to assess such habits and the authors relied on questionnaires to assess the existence of the habit and its severity, no other studies have reported a link to nail biting.

Linge and Linge (1991) reported that finger sucking habits persisting past the age of 7 years may also be a risk to increased resorption during orthodontic treatment while others have implicated tongue thrust (Sameshima and Sinclair, 2004) and tongue pressure (Newman, 1975). Nevertheless the most recent reviews have not identified habits as significant risk factors for OIIRR (Brezniak and Wasserstein, 2002b).

3.3 Systemic and general factors

These are factors that do not directly relate to orthodontic treatment but relate to the general state of the patient with regards to health, gender, age, race and other factors that may alter the physiology of the tissues and thus alter their response to orthodontic forces and possibly change the predisposition to root resorption during orthodontic treatment.

3.3.1 The effect of age

A limited number of clinical experiments have compared the rate of tooth movement in adolescents versus adults (Iwasaki et al., 2008). Some have found that tooth movement was slower in adults than in adolescents (Iwasaki et al., 2004) while others found that

only the lag phase was longer in the adults but once tooth movement entered the third phase there was no difference between the groups (Darendeliler et al., 1997). Similar results were also demonstrated by *Ren et al* on rats (Ren et al., 2003b). They applied a standardized force of 10 cN to the maxillary molars of 6 week old rats versus 9-11 months old rats. They found that there was an initial delay in tooth movement in adult rats but once tooth movement started the rate of tooth movement was the same for the young and older rats.

The views in the literature are not very consistent with regards to the effect of age on the susceptibility to OIIRR. A number of studies have pointed out that age may be a factor in predisposition to OIIRR with older patients being subject to more resorption than younger ones. *Mirabella and Artun* (1995a) found that the mean resorption in adults was not much greater than other studies showed on adolescents but they found that 40% of adult patients showed resorption of 2.5mm or more resorption in one or more teeth. This was higher than the 16.5% reported by *Linge and Linge* (1991) in adolescent patients. Further more a study by *Sameshima and Sinclair* (2001a) found that adult patients had significantly more root resorption than children by as much as 0.8mm only in the lower incisor and canine area but there was no difference in the resorption mean or the mean for the most resorbed tooth when it came to the maxillary incisor area.

However there are a large number of studies that show that age may *not* be as significant in affecting resorption as postulated by the studies above. *Harris and Baker* compared matched groups of adults and adolescents and found that although adults may display

more loss of crestal bone height they did not seem to be at more risk of root resorption (Harris and Baker, 1990). This is in agreement with most recent studies (Hendrix et al., 1994, Harris et al., 2001, Harris and Baker, 1990, Brezniak and Wasserstein, 2002b).

3.3.2 Allergy and immune factors

It is well established that orthodontic tooth movement involves a sterile inflammation within the periodontal ligament with the release of inflammatory mediators and eventually the recruitment of osteoclasts/cementoclasts which then leads to bone/root resorption. So it is logical to expect that factors that may alter the bodies' inflammatory and immune response may play a role in modifying tooth movement as well as root resorption. Patients with allergies or hypersensitivity have an altered or exaggerated immune response to substances and factors that normally do not invoke an immune response (Leite and Bell, 2004). This has led some authors to consider examining the effect of allergy and hypersensitivity on root resorption and how that affects patients' susceptibility to root resorption. *Davidovitch et al* induced allergic asthma in guinea pigs and then applied orthodontic forces to the maxillary molars (Davidovitch et al., 1996). Root resorption was not observed in those teeth as they are free of cementum and continuously erupting but the interesting finding of this study were an increased number of alveolar bone osteoclasts near the areas of compressed PDL over the controls. This may indicate that in asthmatics there may be an increased recruitment of clast cells. In a retrospective clinical study *McNab et al* (1999) reviewed panoramic radiographs of 44 asthmatic patients and 97 healthy patients before and after orthodontic treatment. Asthma

patients were divided into 2 groups medicated and non-medicated, while the healthy patients acted as controls. The controls were age and sex matched so that there was at least two controls per asthmatic patient. The study concluded that asthmatic patients, both medicated and non-medicated, showed more OIIRR in the posterior teeth than controls. This conclusion was drawn after combined tooth analysis was adjusted for treatment factors such as treatment type, extraction and non extraction treatment and treatment duration. It is worth mentioning that when individual teeth were analyzed only the mesial root of maxillary molars exhibited statistical significance to the prescribed ($P<0.003$) and the increased root resorption was limited to mild blunting of the apices. The authors put forward two theories to explain their findings. Firstly they hypothesized that this increased incidence of resorption could be attributed to changes in the immune system. Due to the presence of higher numbers of progenitor cells in the blood (Denburg et al., 1985) and bone marrow (Wood et al., 1998) of asthmatic patients, this may lead to inflammatory mediators entering the PDL at higher levels following the inflammation subsequent to orthodontic loading. They also suggested that the fact that maxillary molars were more affected in the asthmatic group could be due to their proximity to the maxillary sinus. It has been shown that asthma may increase the severity of sinus disease (Dinis and Gomes, 1997) and since some studies have shown (Moskow, 1992, Bauer, 1942) that there is a link between periodontal disease and sinus disease, in which periodontitis may exacerbate maxillary sinus inflammation, this link may also play a role in the opposite direction with sinus inflammation playing a role in the root resorption process. However the authors did not study the incidence of sinus inflammation in their sample and so their hypotheses remain untested.

The question of allergy as a risk factor for OIIRR was also investigated by *Owman-Moll and Kurol* in a somewhat different approach. In their study they loaded maxillary premolars with a buccally directed force in 96 adolescent patients (Owman-Moll and Kurol, 2000). The premolars were then extracted and histologically analyzed for the severity of the resorption. They selected 50 patients which were divided into a group of severe resorption and another of minimal resorption. Several variables were examined as potential risk factors for the increased resorption. They found that allergy was the only variable that showed correlation with increased resorption yet it was not statistically significant. Another recent retrospective investigation from Japan has also reported that allergy, abnormal root morphology and asthma can be considered risk factors for excessive root resorption in Japanese patients (Nishioka et al., 2006).

3.3.3 The role of genetics, heredity and race

A recent review by *Iwasaki et al* discussed the relationship between orthodontic tooth movement and genetics (Iwasaki et al., 2008). To date little research has been conducted to study the influence of genetics on tooth movement. *Iwasaki et al* (2008) described tooth movement as being the phenotype that would be the result of interaction of the patients' unique genotype with the environment and influenced by the clinical factors such as the orthodontic force. Large variations in individual responses have been reported to seemingly similar and controlled clinical variables, point towards an important role of genetic makeup in orthodontic tooth movement (Darendeliler et al., 1997, Iwasaki et al.,

2008, Von Bohl et al., 2004a, von Bohl et al., 2004b). *Iwasaki et al* found the ratio of expression of IL-1β and its receptor antagonist IL-1RA in the GCF as well as IL-1 cluster gene polymorphism are related to velocity of orthodontic tooth movement (Iwasaki et al., 2006). While recent research is pointing towards the important role of genetic control in the velocity of tooth movement more research is needed to identify the complex genes involved to offer a better understanding of the mechanisms involved.

On a similar line of thought several investigators who were trying to test the effects of various orthodontic treatment factors such as the magnitude of force and duration of loading reported that individual variation in response was very variable (Owman-Moll et al., 1996b, Owman-Moll et al., 1996a). This led to the line of thought that may be some individuals are genetically more predisposed to OIIRR then others.

As early as 1975 *Newman* suggested family clustering for OIIRR but the pattern of inheritance was not clear. More recent studies seem to be pointing towards the fact that heredity may be an important factor in the predisposition to OIIRR. Some patients may just be at more risk because of their genetic makeup. *Harris et al* concluded that familial factors play a significant role in the susceptibility to root resorption (Harris et al., 1997). They studied a sample 103 siblings treated by the same orthodontist using the same technique. They found that heritability rates were quite high and could explain about 70% of the variation in resorption. They suggested that studying the underlying biochemical factors is necessary to examine what genes are involved and the mechanism of their expression. Similar conclusions were drawn from a small twin study conducted by *Ngan*

et al using 16 pairs of monozygotic twins and 10 dizygotic twins (Ngan et al., 2004). The study used concordance and heritability estimates to determine the genetic contribution to root resorption. Their results also indicated a genetic contribution but due to small sample size the study could not draw definite conclusions. In a series of studies *Al-Qawasmi and coworkers* examined the genetic contribution to root resorption closely looking at both human and animal models (Al-Qawasmi et al., 2004, Al-Qawasmi et al., 2006, Al-Qawasmi et al., 2003a, Al-Qawasmi et al., 2003b). They managed to find a definite genetic contribution to susceptibility and resistance to root resorption. One study on three inbred strains of mice they found that some strains were more susceptible to OIIRR than others when orthodontic force and environmental factors were standardized this indicates that susceptibility to root resorption is a genetically influenced trait (Al-Qawasmi et al., 2006).

In two human studies the group analyzed the genetic linkage of two different aspects of the resorption process, namely the expression of proinflammatory mediators and also the role of the factors that control osteoclast/cementoclast differentiation and cementum formation.

The first study examined the polymorphisms in genes for proinflammatory mediators IL-1α and IL-1β which are IL-1A and IL-B respectively as well as the gene for IL-1ra which acts as a receptor antagonist (Al-Qawasmi et al., 2003a). They based their selection of those genes based on the close linkage found between IL-1 and bone resorption. Three lines of evidence supported their hypothesis. Firstly polymorphisms in those genes have

been associated with advanced adult periodontitis (Kornman et al., 1997). Secondly the expression of IL-1 in the PDL during orthodontic tooth movement (Davidovitch, 1991) further implicates them with the resorption process with increased levels of IL-1β being detected in the gingival cervicular fluid (GCF) of teeth undergoing orthodontic movement (Grieve et al., 1994). Thirdly variations in the level of IL-1 expression in patients have been implicated in the various responses with regards to the rate of tooth movement and possibly root resorption (Iwasaki et al., 2001a). The study used the transmission disequilibrium test (TDT) which involved the analysis of the DNA material of one affected subject and his or her parents. They used a total of 118 subjects with 73 siblings and 45 parents. At least two siblings from each selected family had orthodontic treatment. Root resorption was assessed on pretreatment and post treatment radiographs. The study found a definite linkage between the IL-1 gene polymorphisms and increased susceptibility to root resorption. Although the linkage was not a 1:1 relation the study found that a reduced expression of IL-1β was associated with more resorption. The explanation offered by the authors was the hypothesis that since IL-1 cytokines are important for bone resorption the reduced expression means that alveolar resorption in response to orthodontic loading is slower, which means the root surface is subjected to prolonged loading leading to more resorption on the root surface.

The second study by the same group (Al-Qawasmi et al., 2003b) examined the effects of two other genes the first was TNFRSF11A which encodes receptor activator nuclear factor Kappa B (RANK). RANK together with its ligand (RANKL) mediates the signaling which leads to osteoclast differentiation and osteoclastogenesis (Nakagawa et

al., 1998) and, in the same manner, cementoclastogenesis (Low et al., 2005). The second gene is tissue non-specific alkaline phosphatase (TNSALP), which plays an important role in cementum formation and mineralization (Beertsen and Van den Bos, 1991). Using very similar methodology to their first study they found a link between D18S64, which is a locus closely related to TNFRSF11A, and OIIRR. From their two studies the authors concluded that susceptibility to root resorption is closely related to genetic factors although it is a multi gene phenomenon and further investigation is required to locate other genetic loci that may contribute. It seems that in the future genetic testing may be applied as a screening method for susceptibility to OIIRR.

Ethnic or racial differences in incidence of root resorption can also be attributed to the difference in the genetic makeup. *Sameshima and Sinclair* (2001a) in a study of 868 patients from six different practices found that Asian patients seemed to show less root resorption than Hispanics or white patients. There are not many studies that support interracial difference in susceptibility to OIIRR. A multi center study by *Smale et al* (2005) which included data from three different centers across the world did not report any racial differences.

3.3.4 Calcium metabolism and vitamin D deficiency and the role of bone turnover

Another interesting factor in the susceptibility to root resorption is alterations in the patients' physiology. Several authors have suggested metabolic and hormonal changes to play a role in altering the bodies' response to orthodontic loading. One such avenue is the

alteration in calcium metabolism. A very good example to that is the case of lactation and associated calcium deficiency. Lactation poses a challenge to the body's calcium homeostatic mechanism especially if associated with a calcium deficient diet (Wong et al., 1980). It has been shown that lactation if coupled with a calcium deficient diet will predictably reduce the bodies bone mineral stores (Rasmussen, 1977). The body compensates for the reduced blood calcium levels by increased secretion of parathyroid hormone (PTH) which in turn stimulates osteoclastic activity and mobilizes calcium from the bone to the blood to compensate. If calcium deficiency is allowed to persist for extended periods of time it can lead to the development of secondary hyperparathyroidism (Midgett et al., 1981). Hypocalcaemia produced by this manner also stimulates the synthesis of vitamin D metabolites (Rader et al., 1979). Increased osteoclastic activity in this manner can lead to osteoporosis and reduced bone density. This can have implications to orthodontic tooth movement and root resorption since they are also clast cell mediated. *Roberts* also showed that in rats stimulated with PTH there is an increased number of osteoclast differentiation in the PDL (Roberts, 1975). *Goldie and King* investigated whether that would also mean a concomitant increase in root resorption with orthodontic loading (Goldie and King, 1984). Their study was designed to compare the tooth movement and root resorption in lactating, calcium deficient female rats with non-lactating female controls on a normal calcium balanced diet. The maxillary first molars were loaded with a mesially directed force of 60 grams. Their results showed that the calcium deficient experimental group experienced greater tooth movement while at the same time maintaining less root resorption. The study also confirmed that the experimental group did in fact have less bone density than the control group which is

consistent with the increased secretion of PTH. The authors explained their findings by the fact that reduced alveolar bone density facilitates more rapid alveolar bone remodeling and so faster tooth movement. This in turn will favor bone remodeling and resorption in response to orthodontic force over root resorption. These findings are also in agreement with those of *Engstrom* (1988) and *Verna et al* (2003) who found that rats with reduced bone turnover rate showed increased root resorption.

3.3.5 Hormones

In addition to parathyroid hormone bone resorption is also regulated by the thyroid hormone 1-thyroxine. It has been documented that patients with hyperthyroidism have increased bone resorption (Adams et al., 1967) while it was also demonstrated that administering high doses of thyroxin to rats increased bone resorption (Adams and Jowsy, 1967). *Shirazi et al* have demonstrated that increased levels of thyroid hormone can significantly increase the rate of tooth movement. It is thought that it increases the efficiency of bone remodeling (Shirazi et al., 1999).

The role of endocrine disturbances in root resorption has been proposed as early as the 1930s when *Becks and cowworkers* found that a large percentage of their examined patients exhibited hypothyroidism. They based their results on clinical observations and measurements of the basal metabolic rate of those patients (Becks, 1939, Becks and Cowden, 1942). In twenty six patients that exhibited excessive root resorption they found low basal metabolic rate to be prevalent. Their observations have not been confirmed by

others such as *Tager* (1951) who was critical of the use basal metabolic rate due to its high variability. In his sample of over 100 cases exhibiting difficulties in orthodontic treatment such as root resorption, delayed dental development and slow response, the calcium metabolism appeared normal ruling out the possibility of hyperparathyroidism. On the other hand 10% of their sample exhibited various degrees of hypothyroidism. They also observed that when treating the hypothyroidism with thyroid hormone supplementation the root resorption progression seemed to halt. In contrast *Carpol* challenged those findings with his findings on a group of 54 patients with hypothyroidism, found no difference in root resorption between those patients and a control group of 50 healthy individuals (Carpol, 1961). He concluded that hypothyroidism does not play a role in the susceptibility to root resorption. His result are somewhat unrepresentative of the problem at hand since the previous studies reported increased resorption in hypothyroid patients after orthodontic loading, his study did not include any orthodontically treated cases and thus the study does not answer the question at hand. More recent studies have examined the relation between the thyroid hormone and OIIRR. *Poumpros et al* studied the effects of low dose L-thyroxin administration on OIIRR in rats (Poumpros et al., 1994). They compared root resorption between controls (orthodontic loading without L-thyroxin) and experimental group (orthodontic force with L-thyroxin) as well as a normal group without appliances. They found the L-thyroxin group exhibited 50% less resorption than the control group. They also found that the thyroxin group showed increased serum alkaline phosphatase activity, which is an indicator of an alteration in the bone metabolism. The authors then went on and used low doses of thyroxin in three patients undergoing orthodontic treatment who they classified

as “high risk” cases for OIIRR (Loberg and Engstrom, 1994). They claimed that no further resorption occurred in those patients and that the patients suffered no clinical side effects to the hormone. The thyroxin was stopped at the completion of orthodontic treatment. They concluded that thyroxin seems to lower the incidence of root resorption. In his commentary on the article *Christiansen* (1994) postulated that thyroxin itself increases the rate of alveolar bone resorption and thus indirectly lowers the amount of root resorption. He also warned against the potential side effects of such hormonal therapy warning about possible reduction in bone density and osteoporosis like symptoms considering human application of this therapy for root resorption to be premature.

The definition of the so called “high risk” patients was challenged by Owman-*Moll and Kurol* (Owman-Moll and Kurol, 1998b). The exact mechanism by which the thyroxin works to prevent root resorption was not explained. It was suggested that it either renders the cementum surface of the root more resistant to resorption or that it could act indirectly by allowing more efficient alveolar remodeling thus reducing root resorption (Brezniak and Wasserstein, 2002a). *Rossi et al* investigated the response of human monocytes to the thyroid hormone T4 and thyrocalcitonin in terms of the production of IL-1β and TNFα (Rossi et al., 1996). Cytokines IL-1β and tumor necrosis factor alpha TNFα which are produced by monocytes are important factors in the response of the PDL to orthodontic loading (Saito et al., 1991). They harvested monocytes from two groups that had completed orthodontic treatment one showing severe root shortening and the other showing little if any resorption. They hypothesized that monocytes from the root resorption subjects would respond to the hormones by production of IL-1β and TNFα and

the ones from the root resorption subjects would respond with greater production of IL-1β and TNFα. They found no difference between the two groups with regards to any of the cytokine parameters.

It is generally accepted that thyroid hormone mediates its action by the interaction of T3 with its local nuclear receptor in the target tissue. Thyroid hormone T4 is considered a prohormone for T3 neogenesis at the local tissue level by process of deiodination, this is mediated by enzymes known as deiodinases. These enzymes have been known to mediate and control the effects of the thyroid hormones on the tissue specific level (Vazquez-Landaverde et al., 2002). In a further study by *Vazquez-Landaverde et al* (2002) it was found that orthodontic loading increased local thyroid hormone production T3 from its prohormone T4 by process of deiodination in the PDL. This occurred without systemic changes in the levels of the hormone indicating that local changes the environment due to the stress of the orthodontic forces changes the local requirements for T3. It also indicates that local remodeling is accompanied by changes in T3 levels. It still remains unclear as to what regulates this change in hormone levels at the PDL site. The same study also found that when exogenous thyroid hormone was administered this activity was even greater. They also found that the rats that were given the exogenous thyroid hormone exhibited significantly less root resorption than controls, which reinforces previous findings as to the protective effects of this hormone on root resorption. A further finding of the study was that the protective effect of thyroid hormone on root resorption was maintained with oral as well as parenteral administration of the hormone.

Although thyroid hormone replacement may seem as an attractive option to facilitate orthodontic tooth movement and to protect against OIIRR it is unlikely that such therapy can be widely applied to orthodontic patients. Thyroid hormone replacement will probably be limited to those patients who suffer a thyroid hormone deficiency of some sort but for the majority of the population this type of hormonal therapy can have serious side effects that far out weigh the benefits of faster tooth movement and risk of root resorption. Increased thyroid hormone levels can induce what is termed "Subclinical hyperthyroidism" which exerts many significant effects on the cardiovascular system as well as the health of the skeleton. It is usually associated with an increased heart rate and a higher risk of supra-ventricular arrhythmias. It is also associated with reduced cardiac performance on effort and decreased exercise tolerance. These abnormalities usually precede the onset of a more severe cardiovascular disease, thus potentially contributing to the increased cardiovascular morbidity and mortality. In addition subclinical hyperthyroidism may accelerate the development of osteoporosis and hence increased bone vulnerability to fractures, particularly in postmenopausal women (Biondi et al., 2005, Uzzan et al., 1996).

4 Long term prognosis of teeth with OIIRR

Although almost all orthodontic patients will suffer some degree of OIIRR it may not necessarily mean an increased risk of tooth loss in the future. Several studies have found that, even in the severe cases of OIIRR, tooth loss may not be a problem provided a healthy periodontium is maintained, however increased mobility may become a problem (VonderAhe, 1973, Sharpe et al., 1987, Remington et al., 1989, Parker, 1997, Levander and Malmgren, 2000, Jonsson et al., 2007).

In a long term follow up study by *Remington et al* they examined 100 patients who had exhibited OIIRR during orthodontic treatment at a mean of 14 years post treatment (Remington et al., 1989). They found that in the majority of cases no adverse effects could be found with the exception of only two cases that showed hypermobility. This was also in agreement with an earlier study by *VonderAhe* (1973) who examined 57 cases with various degrees of OIIRR an average of 6.5 years post retention and found no cases of hypermobility or other adverse effects.

Although the above studies show no real adverse effects of OIIRR several studies have tried to assess at what level the amount of resorption endangers the longevity of the tooth. In another long term follow-up study *Levander and Malmgren* found a significant correlation between tooth mobility, total root length and intra alveolar root length (Levander and Malmgren, 2000). They found that there is an increased risk of tooth mobility if an upper incisor has OIIRR that results in a root that is 9mm long or less.

Similar findings were also reported in a recent study by *Jonsson et al* that used the periotest to assess for tooth mobility 10-25 years after treatment (Jonsson et al., 2007). Although these articles give some quantification as to how much resorption can be considered undesirable it would have been more applicable to describe the root length as ratio to the crown length. It is also not clear as to what the situation is if a patient started off with a root that was 9 or 10 mm long initially. The articles also fail to recognize the possibility of a small clinical crown in which case a tooth with 9mm root length may still have a favorable crown to root ratio.

It should be noted that a reduction in root length has been reported to be less detrimental than an equivalent loss of periodontal attachment especially for cases with 3mm or less resorption (Lupi et al., 1996).

5 Effect of medication on tooth movement and root resorption

Any pharmacologic agents and nutritional supplements consumed by the patient can reach the periodontal tissues through the circulation and thus interact and influence the cells and molecules altering their response to orthodontic forces. The drugs may have the effect to potentiate or inhibit tooth movement as well as exacerbate or reduce root resorption. The effect of several medications on tooth movement and root resorption has been extensively studied (Krishnan and Davidovitch, 2006a).

5.1 Prostaglandins

Prostaglandins are very potent inflammatory mediators and play a role in the inflammatory reaction in the PDL following orthodontic force application. Prostaglandins have been linked with bone resorption as well as with bone apposition (Krishnan and Davidovitch, 2006b). It has also been demonstrated that local injection of prostaglandins increases the rate of tooth movement both in humans and in animals (Yamasaki et al., 1980, Yamasaki et al., 1982, Yamasaki et al., 1984).

Several investigators have examined the role of prostaglandins in root resorption. *Brudvik and Rygh* injected Prostaglandins in the gingival tissues mesial to the maxillary first molars of Wistar rats (Brudvik and Rygh, 1991). The molars were moved mesially with a coil spring and injections were made at 0,3,5 and 7 days. There was no statistical difference in root resorption between the injected and the control side but the

prostaglandin side had a tendency to have more resorption. In a more comprehensive study *Leiker et al* used different concentrations of prostaglandins and injected at various frequencies with orthodontic loading to the maxillary first molar in Sprague-Dawly rats (Leiker et al., 1995). They also found that prostaglandin injection increased the rate of tooth movement while increasing the root resorption. The increased concentration of the prostaglandins and the frequency of injection did not seem to affect the rate of tooth movement but it significantly increased the amount of root resorption. Similar findings were also reported by *Boekenoogen et al* (1996).

5.2 Non steroidal anti-inflammatory drugs NSAIDs

In the same mechanism that inflammatory mediators play an important role in orthodontic tooth movement and root resorption it is possible that anti inflammatory agents that may alter or interfere with the inflammatory process will have an effect on tooth movement and root resorption. Several studies have investigated the effect of short and long term administration of anti-inflammatory drugs on orthodontic tooth movement (Arias and Marquez-Orozco, 2006, de Carlos et al., 2006, de Carlos et al., 2007).

NSAIDs act by inhibiting the production of prostaglandins, this is done through their inhibition of the cyclooxygenase enzyme (COX). COX exists in two forms. The first is COX-1 which is involved in many tissues and releases prostaglandins that are responsible for normal cellular activity such as the synthesis eicosanoids that play an important role in homeostatic function in the gastric mucosa and platelets. COX-2 on the other hand is

induced by proinflammatory mediators and releases prostaglandins involved in inflammation and pain signaling (Bensen, 2000).

Conventional NSAIDs such as aspirin and ibuprofen cannot selectively inhibit COX-1 or COX-2 but inhibit both, which is one of the reasons for their unwanted side effects such as gastric irritation. Acetaminophen also known as paracetamol on the other hand is analgesic only with no anti inflammatory effects and acts centrally rather than on peripheral inhibition of COX (Flower, 2003, Aronoff et al., 2006). *Arias and Marquez-Orozco* found that NSAIDs aspirin and ibuprofen reduce orthodontic movement by reducing the number of osteoclasts due to the inhibition of prostaglandin secretion. Acetaminophen however had no effect on tooth movement (Arias and Marquez-Orozco, 2006).

Selective COX-2 inhibitors have the advantage of relieving pain and having a strong anti-inflammatory effect while avoiding gastric irritation caused by conventional NSAIDs (Flower, 2003). Because conventional NSAIDs have the potential to reduce tooth movement several studies have investigated the possibility of using selective COX-2 inhibitors in attempt to relieve pain associated with orthodontic treatment without inhibiting tooth movement. *De Carlos et al* investigated the effect of a selective COX-2 inhibitor rofecoxib compared with conventional NSAID diclofenac on tooth movement in rats (de Carlos et al., 2006). They found that both inhibited tooth movement but inhibition was only partial in the case of rofecoxib. Nevertheless not all COX-2 inhibitors are the same and so the same researchers compared the effects of three different COX-2

inhibitors on orthodontic tooth movement. They compared rofecoxib, celecoxib and parecoxib. Their results showed that while rofecoxib inhibited tooth movement celecoxib and parecoxib did not with celecoxib showing the least effects on tooth movement and thus the authors recommended the use of celecoxib to control pain associated with orthodontic treatment.

The effects of NSAIDs on root resorption have also been studied. *Villa et al* conducted a human trial using nabumetone, which is a NSAID, to test effectiveness in reducing the amount of root resorption with orthodontic intrusion in human premolars (Villa et al., 2005). The study found that the administration of nabumetone significantly reduced the amount of root resorption while not significantly impeding tooth movement. It caused a decrease of only 0.13mm per month. Celebrex, the commercial name for celecoxib, is a selective COX-2 inhibitor and is a NSAID used for various reasons with the advantage of avoiding gastric irritation and bleeding problems. *Jerome et al* in an animal study on rats found that administering Celebrex in rats during orthodontic tooth movement may offer some protection against root resorption (Jerome et al., 2005). The authors recommended using Celebrex in association with orthodontic treatment not only to reduce pain with appliance activation but also to offer some protection against OIIRR.

The biggest disadvantage of NSAIDs is that long term use can have serious side effects such as gastric irritation and more seriously gastric ulcers and perforations (Flower, 2003). This is why they are usually limited to periods following appliance activation but

recommending them for long term use through out the course of orthodontic treatment to prevent root resorption may not be practical.

5.3 Corticosteroids

Corticosteroids are widely used to treat many conditions including allergies, asthma and other conditions mainly due to their potent anti inflammatory effects. Increasing numbers of people in the modern world suffer from allergic diseases and so it is common to encounter orthodontic patients that are in treatment with corticosteroids whether inhaled or orally administered. Several authors have investigated the effect these drugs may have on bone metabolism, tooth movement and also root resorption. Several studies have shown that corticosteroids may slow down orthodontic tooth movement (Ashcraft et al., 1992, Kalia et al., 2004). Their effect on root resorption on the other hand has been somewhat controversial. Relatively large doses (15mg/kg) used acutely with orthodontic forces on rabbits showed significantly more root resorption than controls (Ashcraft et al., 1992) while in another study the opposite was demonstrated with small doses (1mg/kg) (Ong et al., 2000).

The effect of acute and chronic corticosteroid administration was compared by *Verna et al* on rats (Verna et al., 2006). They found that acute administration of corticosteroids may increase the risk of OIIRR while chronic administration did not seem to be different to the controls without medication. This may be due to the fact that corticosteroids may reduce or inhibit osteoblastic activity, by increasing the blastic cycle with more osteoid

being present that cannot be resorbed by osteoclasts, while enhancing not changing clastic activity. This would favor more root resorption. The authors then advised that in cases where a patient starts an acute course of corticosteroids it may be advisable to go into a passive phase in the orthodontic treatment or, if active treatment is to be continued, to closely monitor for root resorption with periodic radiographs.

5.4 Bisphosphonates

Bisphosphonates are well known potent inhibitors of bone resorption. They are used in many bone and metabolic disorders such as Paget's disease of bone, osteoporosis and hypercalcaemia incident to malignancy. These conditions are characterized by increased bone resorption and bisphosphonates are used in order to reduce or inhibit the resorption process. The exact mechanism of action is not completely known to date but there are several documented mechanisms for their action (Igarashi et al., 1994). They are characterized by a P-C-P structure instead of a P-O-P structure of organic pyrophosphate making them more resistant to hydrolysis by enzymes and giving them high affinity to calcium phosphate crystals (Igarashi et al., 1994). It is worth mentioning that there are several bisphosphonates that have been developed and the mechanism of action can differ from one to the other (Igarashi et al., 1996). Nevertheless they mostly have an inhibitory role on bone metabolism and osteoclastic function. Several studies have examined the possible effects of bisphosphonate administration on orthodontic tooth movement as well as root resorption (Alatli et al., 1996, Engstrom, 1988, Igarashi et al., 1996, Igarashi et al., 1994, Liu et al., 2004). (Igarashi et al., 1994) used a very potent bisphosphonate

AHBuBP administered systemically on a daily basis to rats. They conducted two experiments; in the first experiment they examined the effect of the drug on the rate tooth movement. The maxillary first molars were loaded with a buccally directed force using a standardized spring, and medication was administered every other day. The amount of tooth movement was compared with a control non drug group. They found administration of bisphosphonates significantly inhibited orthodontic tooth movement and it also inhibited the associated root resorption. In the second experiment they examined whether it would also inhibit tooth movement in relapse and so the molars were loaded with the same buccal force and then when the springs were removed the bisphosphonate was administered. They showed significant reduction in relapse as well. Histological examination revealed that fewer osteoclasts and odontoclasts appeared on the alveolar bone and root surfaces respectively. And the authors suggested that bisphosphonates acted on the alveolar bone by altering the recruitment as well as the function of osteoclasts. The same experiments were also conducted using topical administration of bisphosphonates with similar results (Igarashi et al., 1996). This is very encouraging with regards to orthodontic application of these drugs as topical application would suggest less systemic side effects and would affect the bone and roots locally.

In a further study the group examined the effect of topical administration of biphosphonates on root resorption and root resorption repair (Igarashi et al., 1996). From the previous experiment (Igarashi et al., 1994) it was demonstrated that in addition to inhibiting clastic activity bisphosphonates also inhibited or significantly reduced oeteoblastic activity on the tension side of the alveolar bone. The study investigated

whether they would also have an effect on the reparative process of root cementum subsequent to OIIRR. The study used a similar experimental set up as their previous study by *Igarashi et al* (1994). The authors reported that topical administration of Risedronate, the Bisphosphonate used in this study, significantly inhibited OIIRR but did not have any inhibitory effect on the repair process with cementoid apposition in the root resorption areas being unimpaired. The mechanism of action for the prevention of root resorption may be slightly different to that of bone resorption considering the number of odontoclasts was unchanged. It was suggested that the function of the odontoclasts was only affected and not the differentiation. Although the authors suggested that their findings indicate that topical application of Risedronate may be used to prevent root resorption it is still not possible to direct the action of the drug to specifically inhibit the root resorption process without also inhibiting tooth movement and so it is still questionable whether it can be used for that purpose in orthodontics. On the other hand it may prove useful in the prevention of inflammatory root resorption incident to tooth replantation following trauma when orthodontic movement is not necessary.

5.5 Doxycycline

Mavragani et al hypothesized that low doses of systemic doxycycline may have an inhibitory effect on OIIRR (Mavragani et al., 2005). Doxycycline is a chemically modified analogue of tetracycline which is a broad spectrum antibiotic used widely as an adjunct to combat periodontal disease. This was based on the premise that aside from their antimicrobial properties they also showed anti-inflammatory properties (Golub et

al., 1998). Tetracyclines have been shown to inhibit matrixmetalloproteinases such as collagenase and so reduce or prevent the breakdown of collagen (Golub et al., 1990, Golub et al., 1994). They have also been shown to reduce osteoclastic activity and root resorption following flap procedures in rats (Grevstad, 1993). Furthermore *Cvek et al* have shown that tetracycline treatment reduced inflammatory root resorption following reimplantaion of teeth in monkeys (Cvek et al., 1990).

Mavragani et al (2005) conducted an animal to study to examine the effects of low dose systemic administration of doxycycline on root resorption and tooth movement with orthodontic loading in the rat model. They found that although it did not affect the rate of tooth movement it did have an inhibitory effect on root resorption with the experimental group showing less root resorption than the controls. They suggested that doxycycline given in low sub-antimicrobial doses can be used to reduce the risk of OIIRR but more studies on the effects on orthodontic tooth movement need to be conducted before it becomes clinically applicable.

It is worth noting that although treatment with doxycyline as an adjunct to periodontal therapy is approved by the Food and Drug Association (FDA) it is not without side effects. Several studies have reported gastro intestinal disturbances as well as the development of tetracycline resistant microbial strains (Thomas, 1995, Thomas et al., 2000) as potential side effects which makes this option a questionable option for wide use as a preventive measure to OIIRR (Ciancio and Ashley, 1998).

5.6 Effect of fluoride

Some evidence suggests that fluorides may play a role in increasing the root surface resistance to OIIRR. The role of fluorides in rendering tooth enamel more resistant to acid attack by cariogenic bacteria is well established in the dental literature (Burt and Fejerskov, 1996). It has been shown that fluoride is incorporated in all calcified dental structures including cementum and dentin as well as bone (Robinson et al., 1996). Furthermore animal and human research has demonstrated that fluoride is at the highest concentration in cementum when compared to other calcified tissues (Ishiguro et al., 1994, Kato et al., 1990). *Foo et al* investigated whether fluoride administration may also reduce the amount of root resorption with orthodontic loading on rats (Foo et al., 2007). The study found that there may be a tendency towards less resorption with high levels of fluoride but it was not statistically significant. The clinical application of that study is probably limited considering that the levels of fluoride used in the study would be considered highly toxic for humans; the recommended concentration for humans is 0.9 ppm in drinking water. It is also unlikely that the systemic administration of fluorides will be prescribed to orthodontic patients to reduce root resorption considering the serious medical side effects (Robinson et al., 1996). On the other hand this could have relevance to patients who are exposed to high levels of fluorides in their environment or those who suffer from dental fluorosis the question of whether those patients may be more resistant to OIIRR is still unanswered.

6 Glucosamine and Chondroitin Sulfate

Recently nutritional supplements, also called “nutraceuticals”, glucosamine (GS) and chondroitin sulfate (CS) have been introduced in the management of OA and related symptoms. They have been ranked the third best selling nutritional supplements in the US from 1997-2000 with over 300 million dollars in sales in the year 2000 alone (Biggee and McAlindon, 2004a). It is estimated that around 5-8% of US adults use the supplements (Marra, 2002).

The term 'nutraceutical' was coined from 'nutrition' and 'pharmaceutical' and was originally defined as “a food (or part of the food) that provides medical or health benefits, including the prevention and/or treatment of a disease” (Kalra, 2003).

GS and CS have been reported to relieve pain associated with osteoarthritis as well as aid reduction of cartilage and joint degeneration but the exact mechanism of action remains unresolved.

Osteoarthritis (OA) is a chronic disease characterized by irreversible damage to joint structures. This includes loss of articular cartilage, formation of osteophytes, alterations in the subchondral bone and synovial inflammation (Monfort et al., 2008). Osteoarthritis can seriously impair the patients health related quality of life with pain and functional disability being the major complaints.

It is estimated that around 33% of adults in the United States suffer from arthritic or rheumatic conditions (Centers for Disease Control and Prevention, 2001) with the cost of care over 22 billion dollars in 1995, with a total loss of 82 billion dollars when loss of productivity is also considered (Praemer et al., 1999).

Several pharmaceutical agents have been trialed in the treatment of OA with NSAIDs being among the most widely used. Recently GS and CS have been introduced with success in management of OA. They belong to a category of compounds that have a slow acting symptomatic effect in OA and so were termed “symptomatic slow-acting drugs for OA” (SySADOA) (Monfort et al., 2008). Most of the compounds suggested as SySADOA are naturally occurring in the body and articular tissues. GS and CS are substances found naturally in the body. GS is a form of amino sugar that is believed to play a role in cartilage formation and repair. CS is part of a large protein molecule (proteoglycan) that gives cartilage elasticity.

6.1 Glucosamine

Glucosamine is an amino monosaccharide composed of glucose and a bound amino group. Proteoglycans form the extra cellular matrix of cartilage as well as many other connective tissues of the body including the periodontal ligament. Glucosamine is a monosaccharide, which is part of a larger group of molecules glycosaminoglycan GAG that is incorporated into the cartilage proteoglycans (Lozada, 2007). Glucosamine is formed naturally in the body and has recently been made available as a nutritional

supplement mainly for the treatment of OA (Biggee and McAlindon, 2004b). It is believed that glucosamine plays a role in cartilage formation and repair, although the exact mechanism of action remains unknown (Biggee and McAlindon, 2004b).

6.1.1 Pharmacokinetics

There are several forms of glucosamine supplements mostly derived from bovine or shellfish chitin. Recently production from a vegetarian source, using a process of microbial fermentation of corn-derived glucose, has been introduced both for efficiency of production and to reduce the risk for patients with shellfish allergies (Almada, 2003).

Glucosamine is commercially available in two forms; glucosamine hydrochloride and glucosamine sulfate (GS). Glucosamine hydrochloride is considered more stable but glucosamine sulfate (GS) may have more biological efficacy as sulfate is also a constituent of cartilage matrix (Bruyere and Reginster, 2007). Furthermore GS has been more widely used and more extensively studied on animal models (Barnhill et al., 2006).

It is difficult to study the pharmacokinetics of glucosamine considering it is an endogenous substance that is quickly utilized by the body (Adebowale et al., 2002). In a series of studies, using radioactive labeled glucosamine, *Setniker and co-workers* studied the pharmacokinetics of glucosamine in rats, dogs and humans (Setnikar et al., 1986, Setnikar et al., 1991, Setnikar et al., 1993). It was found that glucosamine rapidly diffuses to the tissues of the body with special tropism to the articular tissues and bone. They

studied intravenous, intramuscular and oral administration and found that the oral route provides sufficient bioavailability with 87% of the orally administered dose absorbed into the plasma. Those results were also in agreement with the findings of *Adebowale et al* (2002) on dogs.

6.1.2 Mechanism of action

Several mechanisms of action have been proposed for glucosamine on cartilage. Originally it was thought that the compound only provided the building blocks for cartilage and acted as an exogenous source for cartilage matrix components (Setnikar et al., 1991). Research has also demonstrated that it can normalize cartilage metabolism as well as reduce cartilage degeneration (Lippiello et al., 2000). Invitro studies have shown that glucosamine can stimulate the synthesis of cartilage GAGs and proteoglycans (Bassleer and Franchimont, 1998). It has also shown some anti inflammatory properties, although more effectively if combined with CS, as it was demonstrated to reduce nitric oxide production by human chondrocytes (Shikhman et al., 2001).

Glucosamine is commonly formulated with other supplements such as vitamin C, manganese and chondroitin sulfate CS (Biggee and McAlindon, 2004a). Nevertheless it is most widely used in combination with chondroitin sulfate CS.

6.2 Chondroitin Sulfate (CS):

CS is a major component of many of the bodies' connective tissues including cartilage, bone, tendons, ligaments and skin (Monfort et al., 2008). It is a sulfated glycosaminoglycan that is composed of a long unbranched polysaccharide chain with the repeating disaccharide structure of N-acetylgalactosamine and glucuronic acid. N-acetylgalactosamines are mostly sulfated usually in position 4 and 6 (CS4 and CS6) making it a strongly charged polyanion. CS is believed to play a major role in imparting the articular cartilages' ability to resist stresses during various loading conditions by providing it with resilience and elasticity (Monfort et al., 2008).

6.2.1 Pharmacokinetics

CS can be obtained from animal or marine cartilage such as bovine, porcine or shark cartilage. Most of the studies have used CS obtained from bovine trachea with 95% purity, which is also the CS used in clinical trials (Barnhill et al., 2006). Naturally occurring CS has a molecular weight of 50-100 kDa which drops to 10-40 kDa after extraction and purification.

Studies on pharmacokinetcs have revealed that CS can be absorbed effectively via the oral route both in human and animal trials and provide sufficient bioavailability with rapid absorption at the gastric and intestinal levels (Monfort et al., 2008). Using

radioactive labeling *Conte et al* found sufficient bioavailability with oral administration(Conte et al., 1991b); this is also in agreement with *Adebowale et al* who showed oral administration to provide sufficient bioavailability for effective pharmacologic effect in dogs when administered orally (Adebowale et al., 2002). Human trials produced similar findings (Conte et al., 1991a). In a study on oral bioavailability performed on healthy volunteers *Volpi* (2002) found an increase of 200% in plasma levels of CS from pre-dose levels with a peak after 2 hours and significant levels from 2-6 hours.

The bioavailability of CS can vary from 15-24 % of the orally administered dose (Monfort et al., 2008) and is found in the plasma as high, low and intermediate molecular weight metabolites (Ronca et al., 1998). 10 % of the absorbed fraction is CS and 90% is lower molecular weight depolymerised derivatives (Monfort et al., 2008).

In the same study by *Ronca et al* (1998) using scintigraphic analysis with radioactive isotopes, CS demonstrated tropism to cartilaginous tissues in rats and knee tissues in humans. It has also been demonstrated to increase in the synovial fluid of joints.

CS is considered to be a slow acting drug SySADOA in the treatment of OA. Its maximal effect is only attained after several months of treatment (du Souich and Verge´s, 2001) and it also has a carryover effect that persists after treatment is stopped (Monfort et al., 2008). Its half life has been estimated to be around 15 hours (du Souich and Verge´s, 2001).

CS is excreted through the kidneys; one study found 19% of the orally administered dose and 53 % of the IV administered dose was excreted through the kidneys within 24 hours after administration (Ronca et al., 1998).

6.2.2 Mechanism of action

CS has several modes of action in treatment of OA. Firstly it has been shown to have an anti-apoptotic effect on chondrocytes. It has been shown that apoptosis of chondrocytes is higher in OA than in healthy subjects (Blanco et al., 1998). Considering that chondrocytes produce the cartilage matrix and regulate cartilage metabolism the number of chodrocytes is important for the maintenance of cartilage. CS was reported to significantly reduce the apoptic index of chondrocytes in mice with OA (Caraglia et al., 2005).

Secondly CS has been shown to increase the synthesis of proteoglycans. It is believed that it provides the building blocks for the synthesis of proteoglycans and increases the sulfate incorporation in OA proteogycans. For this reason it is believed that increasing CS' concentration may account for increased proteoglycan production with its beneficial effects.

CS also reduces the effects of matrix degrading enzymes such as proteases. It is well documented that the turnover of extracellular matrix components is an integral part of tissue remodeling, development and morphogenesis (Bode et al., 1999). Matrix metaloproteases (MMPs) are a particular group of enzymes that play an important role in the remodeling of cartilage, bone and other calcified tissues during physiological as well as pathologic remodeling (Bode et al., 1999). MMPs activity is normally in a balance regulated by its specific inhibitors (TIMPs) (Bode et al., 1999). In OA patients MMPs

activity and the balance with TIMPs is disturbed (Dean et al., 1989). CS has been found to decrease the effects of MMPs by down regulating their synthesis as well as reducing their activity thus accounting for its chondroprotective properties (Monfort et al., 2005, Chou et al., 2005, Holzmann et al., 2006, Chan et al., 2005a).

Last but not least, CS has been found to have anti inflammatory properties. Inflammation and inflammatory mediators and cytokines play an important role in the disease process of OA (Monfort et al., 2008). These include IL-1β, nitiric oxide (NO) and prostaglandin E2 (PGE2). IL-1β is believed to be the principle cytokine responsible for the degradation of extracellular matrix components in OA (Martel-Pelletier et al., 2005). NO on the other hand can induce inflammation as well as cause tissue damage making it particularly important as it not only contributes to the symptoms but also to the disease process. PGE2 is a well known inflammatory mediator that induces pain as well as increases the production of catabolic factors (McCoy et al., 2002). It also potentiates the effects of other inflammatory mediators (McCoy et al., 2002).

Experiments on human articular chondrocytes have shown that CS can significantly reduce the production of IL-1β-induced PGE2. They have also demonstrated that CS reduces the levels of IL-1β-induced extracelluar kinases which may explain some of its anti catabolic effect (Bassleer et al., 1998). Further more CS' anti inflammatory properties are much more pronounced when used in combination with GS (Chan et al., 2005b).

CS has also been found to have effects on the subchondral bone alterations in OA (Monfort et al., 2008). As mentioned previously three factors that influence bone metabolism have been identified namely OPG, RANKL and RANK. The first two are produced by osteoblasts with RANKL being essential for osteoclast differentiation and bone resorption. OPG on the other hand is a decoy receptor that blocks RANKL therefore preventing it from binding with its receptor RANK on osteoclasts thus inhibiting osteoclast differentiation and bone resorption. The effect of CS on these bone resorption factors has recently been studied by *Kwan et al* on human OA sub-chondral osteoblasts after stimulation with vitamin D3 (Kwan et al., 2007). The results showed that CS up-regulated the ratio of OPG:RANKL i.e. it up-regulated OPG and also down-regulated RANKL. Considering that in OA abnormal osteoblasts increase the expression of RANKL and increase bone resorption; CS would have a positive effect on bone protection in OA (Monfort et al., 2008).

6.3 Combination of GS and CS

Most recent studies have concluded that GS and CS sulfate are more potent in the management of OA when used in combination (Monfort et al., 2008). It has been suggested that while GS plays a structure modifying role in the treatment CS plays a symptom modifying role (Monfort et al., 2008). This may be considered an oversimplification considering they both have similar actions and it is difficult to see where the effect of one ends and the second begins (Monfort et al., 2008).

Several studies have demonstrated the increased anti-inflammatory properties of CS when combined with GS. Although CS was shown to reduce PGE2 production, only the combination of GS and CS reverted the levels of IL-1β-induced PGE2 to control levels. The same was for IL-1β-induced NO (Chan et al., 2005b). It has also been demonstrated by the same authors that the combination of GS and CS was more effective in reducing the gene expression for NO synthase, COX-2 and PGE than CS alone (Chan et al., 2006). From the above data it can be extrapolated that GS and CS used in combination are effective in reducing the level of expression of genes involved in inflammatory conditions. This is also supported by recent clinical trials reporting increased effectiveness of the combination in the management of knee OA (Clegg et al., 2006).

6.4 Toxicology

GS and CS are widely used due to their well documented safety profiles. Compared with traditional OA medications such as NSAIDs they do not have any serious side effects when used for long periods of time.

When evaluating the safety of any drug two dosage levels need to be identified; the first is the no observed adverse effect level (NOAEL), which is defined as the highest dose of the compound that did not produce any adverse effects. The second is the lowest observed adverse effect level (LOAEL), which is defined as the lowest dose that produced an adverse effect (Hathcock and Shao, 2007). Thirdly the observed safe level (OSL) or the upper safe limit (ULS) which refer to the highest dose tested that did not

bring forth any adverse effect (Hathcock and Shao, 2007). The OLS and ULS differ slightly in that ULS refers to dose of the compound accounting for any additional quantities that may be in the normal diet. Considering the nature of the raw materials in GS and CS there is little or no dietary intake and so OSL and ULS would be the same if there was little or no dietary intake of the substance (Hathcock and Shao, 2007).

Hathcock and Shao conducted a risk assessment on the use of GS and CS in both animal and human subjects (Hathcock and Shao, 2007). They conducted a wide review of the published literature including clinical trials as well as experimental studies. They found that neither a NOAEL nor a LOAEL could be identified for both substances at any level. Because there was no critical effect OSL method was used to assess the safety of drugs at the maximum prescribed doses. They found that no adverse effects of GS and CS could be identified in the literature. The highest observed intake OSL was 2000mg/day for GS and 1200mg/day for CS in well controlled RCTs without any discernable adverse effects making them confident of their long term safety.

It is still worth mentioning that some concerns were raised with regards to GS causing allergic reactions in people with shellfish allergies. Nevertheless reports of allergic reactions to GS supplement are rare (Tallia and Cardone, 2002), companies are required to label them accordingly and with the introduction of new shellfish free GS from vegetarian sources this may no longer be a concern (Almada, 2003).

Concerns have also been raised around the potential for glucosamine to cause or exacerbate diabetes. These concerns came from the fact that it competes with glucose in the liver for carbohydrate metabolism (Biggee and McAlindon, 2004b). Cell cultures and animal experiments have shown that it may interfere with the transport of glucose and cause insulin resistance (Balkan and Dunning, 1994). On the other hand (Echard et al., 2001) found no risk of increased insulin resistance or other related perturbations in two rat strains highly sensitive to sugar when given GS and CS in combination or separately. This is also in agreement with most long term RCTs who found none of their subjects developed diabetes during the trials (Pavelká et al., 2002, Reginster et al., 2001).

The most commonly reported side effect of GS and CS is limited gastrointestinal upset and some flatulence. This may improve over time and can be eliminated with discontinuation of the medication.

From the above it can be concluded that GS and CS are safe for use in the long term with little or no reported health risks or adverse side effects.

6.5 Relevance to orthodontics

6.5.1 Proteoglycans in the PDL

Proteoglycans such as CS, as mentioned above, are not only present in the articular tissues but they are an integral part of most of the bodies' connective tissues including the PDL, alveolar bone and cementum (Berkovitz, 1990). In the extracellular matrix of the connective tissues of the periodontium collagen makes up 60% of the organic matrix of non-mineralized tissues such as PDL and up to 90% of the organic matrix of mineralized tissues such as the alveolar bone (Waddington and Embery, 2001). The collagenous fibrous network provides structural support and is embedded in and interacting with a non-collagenous matrix that consists of proteoglycans and various glycoproteins (Waddington and Embery, 2001).

Proteoglycans are comprised of a protein core to which one or more glycosaminoglycan (GAG) chains are attached. Both the protein and the type of GAG are important in determining the function within the extracellular environment. GAG chains are linear and consist of a disaccharide repeating unit of hexouronic acid and an n-acetyl hexosamine (Table 1) According to the repeating disaccharide units there are 7 GAG species 6 of which are sulfated these include: chondroitin 6 sulfate (C6S), chondroitin 4 sulfate (C4S), Dermatan sulfate, heparin sulfate and keratan sulfate (Waddington and Embery, 2001).

Molecular size	Name		distribution and function in the PDL
Large molecular size	Large aggregating proteoglycans (CS containing)	Versican	• involved in maintaining tissue hydration/ contribute to the overall structural scaffolding of the extracellular matrix (Bartold and Narayanan, 1998) • mainly soft connective tissues, PDL and gingiva
		Agrecan	
Small molecular size	Small Leucine-rich Proteoglycans (SLRPs)	Biglycan	• Biglycan and decorin carrying DS chains predominate in the PDL • Biglycan and decorin with one or two CS chains predominate in the bone and cementum
		Decorin	
		Fibromodulin	
		Lumican	
Small molecular size	Cell Surface Proteoglycans	Syndecan	• identified on most cell surfaces • Cell-cell and cell-matrix interactions, binding of growth factors and cytokines. • influences cell adhesion proliferation and differentiation

Table 18 modified from (Waddington and Embery, 2001) and (Bartold and Narayanan, 1998)

6.5.2 Function of proteoglycans in the PDL

The structure of proteoglycans has been found to be intimately related to their function but it is important to regard the functions of proteoglycans as groups of functions and not as individual entities (Bartold and Narayanan, 1998). The distribution of proteoglycans within the tissues of the periodontium reflects the function of these macromolecules in the synthesis and remodeling of the connective tissue (Bartold and Narayanan, 1998).

One of the most important proteoglycan families within the PDL are the small leucine-rich proteolclans (SLRPs) (Waddington and Embery, 2001).

The extracellular matrix proteoglycans are principally associated with regulating the physicochemical properties of the tissues (Bartold and Narayanan, 1998). Large proteoglycans such as agrecan and versican are highly charged and thus involved in maintaining tissue hydration contributing to the overall structural scaffolding of the extracellular matrix (Bartold and Narayanan, 1998). Smaller molecules such as the SLRP decorin are believed to play a role in regulating collagen fibril formation. They have also been found to play a role in regulating mineralization. This is supported by the fact that CS was found to be predominating in the SLRPs found in mineralized tissues while DS was predominating in soft connective tissue (Waddington and Embery, 2001). Decorin and biglycan carrying one and two CS chains respectively are two SLRPs predominant in mineralized tissues of the periodontium (Waddington and Embery, 2001). It is thought that this may reflect their potential for inhibiting, controlling or promoting the mineralization process (Embery et al., 1998). Proteoglycans from the SLRPs family such as decorin (CS containing) have also been found to have the capacity to bind and regulate growth factors such as TGF-β (Yamaguchi et al., 1990). This important function provides additional mechanisms for cells to communicate with their environment as well as to regulate growth factor and extracellular matrix expression through feedback loops (Bartold and Narayanan, 1998).

Proteoglycans that are present on the cell surface such as syndecans are believed to play a role in cell-cell and cell-matrix interactions as well as binding a variety of growth factors, cytokines and protease inhibitors. Therefore these proteoglycans influence cell adhesion, differentiation and proliferation (Waddington and Embery, 2001).

6.5.3 Distribution of Proteoglycans in the Periodontium

6.5.3.1 Periodontal ligament

In the extracellular matrix of the PDL DS containing proteoglycans have been found to be predominating (Lajarva et al., 1992). With specific antibody techniques other proteoglycans including CS proteoglycans such as decorin and biglycan have also been identified in lesser quantities (Häkkinen et al., 1993). It has also been demonstrated by cell cultures that PDL fibroblasts can synthesize these proteoglycans (Lajarva et al., 1992).

6.5.3.2 Alveolar bone

The predominant GAG in alveolar bone is CS while DS has also been identified but in lesser quantities (Waddington and Embery, 1991). CS has been detected on the ultrastructural level using immunohistochemical techniques and was found to be located in both the cell surface and around bone canaliculi and osteocytes (Bartold and Narayanan, 1998). Within the mineralized matrix a small CS proteoglycan is also present

(Waddington and Embery, 2001). Versican (large CS containing proteoglycan) has been identified in osteoid. It has been suggested that it plays a major role in the initial formation of the extracellular matrix. It was found to be removed during matrix remodeling prior to the mineralization process (Waddington and S., 1998).

6.5.3.3 Cementum

In cementum CS and DS were identified by *Cheng et al* using immunolocalization techniques (Cheng et al., 1996). The distribution was principally with the cementoblasts and cementocytes. CS proteoglycans were found in relation to cementocytes and on the borders and lumina of lacunae and canaliculi in cellular cementum. They were also identified on cementoblasts on the root surface and in the PDL (Ababneh et al., 1999). It should be noted that they were only expressed by a limited proportion of cementocytes (Ababneh et al., 1999).

6.5.4 Role of Proteoglycans in tooth movement

Extracellular matrix remodeling plays a vital part in orthodontic tooth movement and root resorption. The forces that are applied to the tooth are transmitted to the surrounding tissues of the periodontium. *Kagayama et al* using immunolocalization found a change in the profile of proteoglycans in the PDL with increased detection of Chondroitin-6-sulfate near the bone surface corresponding to areas of compression in the non-hyalinized and hyalinized zones of the PDL, whereas that of CH-4S/DS did not appear to be influenced

by the mechanical stress (Kagayama et al., 1996). Further more, analysis of the gingival cervicular fluid GCF in relation to tooth movement detected chondroitin-4-sulfate in the GCF on the side of the tooth towards which the force was directed (Last et al., 1985). In another study by the same group an increased level of C4S was detected when the teeth were undergoing the most rapid movement in the vertical and horizontal planes (Samuels et al., 1993). No significant increases in C4S were detected in teeth showing smaller horizontal-only or vertical-only movements. Another study on the early stages of orthodontic treatment found increased levels of C4S at 10 weeks of treatment. Further more teeth that showed the greatest extent of movement showed increased C4S levels in GCF until 22 weeks, while C4S levels declined in those teeth moving to a small extent.

Waddington suggested that, since the orthodontic model is a non-plaque, non-disease related process, that the increased levels of CS in the GCF represent biological alterations to the deeper seated periodontal tissues particularly the alveolar bone. On the other hand the absence of DS which is the major GAG in the PDL makes this possibility less clear. Nevertheless these studies suggest that CS in the GCF represents a marker for active alveolar bone and PDL turnover (Waddington and Embery, 2001).

Remodeling of the ECM of the PDL is believed to play an important role in tooth movement. MMPs are a group of enzymes which have been implicated in the remodeling of the ECM. The function of these enzymes is regulated by a number of inhibiting factors TIMPs. It is believed that modifying the function of these enzymes and their inhibitors

can have an effect on tooth movement. A study by *Holliday et al.* (2003) demonstrated that TIMP can inhibit tooth movement.

6.5.5 GS and CS relation to TM and OIIRR

There has not been a study to date that has evaluated the effects of GS and CS on orthodontic tooth movement and root resorption. Although there has been no direct link demonstrated, it may be inferred from studying the effect of GS and CS in OA that they may possibly influence the periodontium and thus tooth movement and root resorption

Firstly it is well documented that inflammation plays an important role in tooth movement and root resorption and GS and CS have been documented to have anti-inflammatory effects which are stronger when they are used in combination.

Secondly GS and CS are proteoglycans not only are they building blocks for the connective tissue matrix of the cartilage but they are an important component of the soft and hard tissues of the periodontium.

Thirdly CS has an effect on inhibiting tissue breakdown by modifying the functions of MMPs the same enzymes are also involved in tissue breakdown and remodeling in the PDL.

Fourthly CS has been demonstrated to play a role in regulation of bone resorption by it up-regulating OPG and also down-regulating RANKL and thus inhibiting bone resorption.

7 Research tools and methodologies

7.1 Methods of measuring root resorption

Several methods have been used in the literature in attempts to assess and quantify OIIRR. These approaches include a variety of two dimensional (2D) methods including the use of conventional intraoral radiographs, extraoral views in the form of lateral head films and panoramic views, conventional light microscopy to scanning electron microscopy. On the other hand three dimensional approaches have also been used which include stereo-imaging using scanning electron microscopy and volumetric measurements using 3 dimensional x-ray micro computed tomography (microCT).

Several retrospective clinical studies investigating the incidence of root resorption and have attempted to quantify OIIRR using standard intraoral periapical x-rays (Linge and Linge, 1983, Linge and Linge, 1991, Mirabella and Artun, 1995b, Mirabella and Artun, 1995a, Remington et al., 1989, Sameshima and Sinclair, 2001b, Sameshima and Sinclair, 2001a). From a practical point of view the use of standard intraoral views or panoramic views makes it easy to conduct large surveys of multiple practices using the standard

records employed by the orthodontist without the need to expose the patients to additional radiation or experimental procedures. This approach has many limitations with regards to the accuracy and reliability of the quantitative data produced. Firstly the x-rays may only be useful in detecting root shortening while surface resorption can only be detected if it lies on the mesial or distal surfaces or at a surface that falls at right angle to the x-ray beam. Further more, even the root shortening measurement is not very reliable considering the variation in magnification and projection errors such as foreshortening and elongation. This becomes even a greater problem in orthodontics considering the crown inclination may be changed considerably with treatment (Chan and Darendeliler, 2004). Some authors (Costopoulos and Nanda, 1996) have tried to standardize intraoral radiographs by the use jigs and positioning devices which does improve the reliability of the measurements but still only measures root shortening, ignoring buccal and lingual resorption which may show more extensive resorption lesions (Chan and Darendeliler, 2004).

The use of panoramic views introduces an even greater error considering that it is not really possible to tilt the beam and change the angulations of the film to accommodate tooth inclination differences and changes. It is also limited by the focal trough and any part of the arch that occurs outside the average trough may not appear on the radiograph, this is of particular importance in cases with skeletal problems such as severe Class IIIs and Class IIs in which incisor inclination may be extreme. In some instances they may not even appear on the film (Chan and Darendeliler, 2004). This was demonstrated well by *Sameshima and Asgarifar* (2001) who compared the accuracy of periapical views with

panoramic views in assessing and quantifying root resorption and found that panoramic views tended to overestimate the amount of resorption by an average of 20%.

Light microscopy after serial sectioning was used by a series of studies from Sweden (Kurol and Owman-Moll, 1998, Kurol et al., 1996, Owman-Moll, 1995, Owman-Moll and Kurol, 1998a, Owman-Moll and Kurol, 1998b, Owman-Moll and Kurol, 2000, Owman-Moll et al., 1995b, Owman-Moll et al., 1995a, Owman-Moll et al., 1996b, Owman-Moll et al., 1996a). Using light microscopy and histological examination may shed light on the cellular and tissue level interactions and changes that play a role in root resorption. Their use in quantifying root resorption was questioned for several reasons (Chan and Darendeliler, 2004). Firstly the microtome was set on 4microns and the tooth was serially step-sectioned along the long axis in a bucco-lingual direction for half the tooth. The other half of the tooth was serially sectioned into three sections mesiodistally in the longitudinal plane. *Chan and Darendeliler* (2004) diagrammatically illustrated how craters can be easily missed when using this method. Secondly because root resorption craters vary greatly in shape and size certain tortuous or C-shaped craters can be missed or miscalculated. Further more the studies used the micrometer mounted on the eyepiece of the microscope to quantify the resorption; this may have led to some parallax errors. The units used for quantification were also arbitrary.

Scanning electron microscopy (SEM) is another method popularly used in the assessment of root resorption (Acar et al., 1999, Barber and Sims, 1981, Harry and Sims, 1982, Kvam, 1972b, Kvam, 1972a). This method offers very detailed views of root resorption

craters and has enabled many researchers to describe root resorption craters and their distribution on the tooth surface with great accuracy. Nevertheless the quantification of root resorption craters which are three dimensional in nature using this two dimensional visualization technique may be somewhat deceptive. Tooth root surfaces are curved which makes a straight on view of the crater not easy to achieve this also introduces a parallax error as has been demonstrated by *Chan and Darendeliler* (2004). The two dimensional images of the craters were also obtained on micrographs which were then pieced together and then the craters were measured with a digitizer. This may have also introduced an error especially for craters at the edges of the micrographs. Due to the two dimensional nature of this imaging technique the true extent of root resorption craters may have not been adequately represented.

Arguably three dimensional imaging and volumetric measurements of the root resorption craters can give a more accurate and representative quantification of the amount root resorption. Initially three dimensional images were obtained from two dimensional images of root resorption craters by using stereo imaging techniques with two dimensional SEM images and then using software to produce a three dimensional view of the crater. This was demonstrated by researchers from Sydney (Chan et al., 2004b) and was then calibrated and its accuracy tested by measuring the volume of pre-calibrated pyramidal indentations using the Vickers hardness tester (Chan et al., 2004a).

Although this method is accurate it is relatively labor intensive and time consuming as well as technique sensitive. Another recently introduced method used extensively by our

department is the use of x-ray micro-computed tomography (mircoCT) to obtain a three dimensional image of the tooth and root structure and then use software to isolate the root resorption craters and calculate their volume. Using the microCT enables a non destructive way of imaging the samples and produces a very high resolution three dimensional image of any calcified structure. Another advantage is that limited or no sample preparation is necessary. Root resorption craters can then be accurately isolated without the limitations of micrographs and thickness of the blades of microtomes employed in microscopy techniques. Isolated craters can then be measured accurately for volume, depth, width as well as surface area. It also permits the detection of various radio-densities allowing the different calcified tissues to be defined in terms of enamel, dentine and cementum. The microCT has been employed by numerous studies in our department to quantify root resorption in both human and animal samples (Barbagallo et al., 2008, Foo et al., 2007, Harris et al., 2006).

7.2 X-Ray Microtomography

7.2.1 History and development

X-Ray microtomography was developed by in 1982 by *Elliott et al* (1982) as a downscaled miniature version of the conventional medical CT scanner introduced ten years earlier by *Hounsfield* (Hounsfield, 1973). X-ray micro tomography could be used to study samples measuring as small as 1mm in diameter with a resolution of 12 microns. This downscaled version has enabled the scanning of very small samples without the

need for an enormous increase in exposure. In medical CT scanning although it is possible to obtain resolution of a fraction of a millimeter, it is unlikely to be possible to improve the resolution much further even with the use of more sensitive detectors. This is due to the fact that in order to double the resolution the exposure needs to be increased by a factor of sixteen. This means that a ten thousand fold increase in exposure is required in order to obtain only a ten fold improvement in resolution. X-Ray microtomography offers the possibility to maintain the signal (attenuation coefficient) to noise ratio, a measure of image quality, with only a thousand-fold increase in exposure for a ten fold improvement in resolution. This is because the specimen size is much smaller which requires X-rays of smaller energies and the measured attenuation coefficients are higher (Davis and Wong, 1996).

Computed Tomography produces a two dimensional map of X-ray absorption and attenuation in a slice of a given sample or subject. This enables there to be no compression of the three dimensional data into a two dimensional plane. The machine obtains a series of X-ray projections made through the slice at various angles around a perpendicular axis. With digital computing it is then possible to combine the series of two dimensional maps into a three dimensional map or image (Davis and Wong, 1996).

In addition to the size and resolution differences another difference between microCT and conventional medical CT is that in conventional CT the beam and the sensor rotate around the subject while in microCT the specimen is placed on a rotating platform while the x-ray source and sensor are fixed.

7.2.2 Application of MicroCT

Several studies have used the microCT in the investigation of bone as well as dental tissue. On the rat model several studies have utilized the microCT to detect structural and density changes (Mechanic et al., 1990, Postnov et al., 2003, Wong et al., 1995). With regards to the dental application numerous studies have used microCT to examine enamel and dentine structure (Anderson et al., 1996, Atar et al., 2007) as well as the three dimensional examinations of carious lesions (Dowker et al., 2003, Dowker et al., 2004, Willmott et al., 2007, Wong et al., 2006). Dental structures of rats have also been described (Atar et al., 2007).

With regards to root resorption, studies from the University of Sydney were the first to use microCT to examine and quantify root resorption (Harris et al., 2006). *Harris and co-workers* scanned human premolars loaded with heavy and light orthodontic forces using microCT scans. They were able to obtain accurate volumetric measurements of root resorption craters and were able to conclude that the volume of resorption was less in light forces than with heavy forces. This method offered an accurate and quantitative tool to assess root resorption in all three dimensions of space. Since then, a number of studies from our department have utilized the microCT in order to quantify root resorption in response to various orthodontic loading regimes as well as the study of the repair process in human premolars (Barbagallo et al., 2008, Harris et al., 2006). Similar methodology has also been used to study root resorption in the rat model by *Foo et al* (2007) who

compared root resorption volumes with orthodontic loading in the mandibular first molar of rats when placed on a fluoride rich diet. It is very difficult to extract rat molars without damaging them and so the authors scanned the entire buccal segment and then digitally isolated the molars from the surrounding alveolar bone.

7.3 The rat model for tooth movement and root resorption

The rat model is among the most popular models used to study orthodontic tooth movement. A systematic review by *Ren et al* (2004) found that between the years 1982 and 2002, 57% of animal research on orthodontic movement was conducted on rats. When data from animal research is interpreted it is always questioned how readily it can be extrapolated to human subjects (Reitan and Kvam, 1971). There are obvious limitations and physiologic differences between humans and animals that make findings sometimes difficult to directly apply to humans. For example the life span of most experimental animals is very short compared to that of humans. The average life span for most rodents is in the range of three years and so temporal data such as periods of appliance activation which seem short may be considered very lengthy when the percentage of the animals' life is considered. In addition the physiologies of the tissues are also different. Rats tend to have denser alveolar bone compared to that of humans with no osteons and the bone plates lacking marrow spaces (Ren et al., 2004). There is also less osteoid on the alveolar bone surfaces than in humans (Reitan and Kvam, 1971). It has also been reported that rat bone has very little acid mucopolysaccharides which may account for its calcium content (Reitan and Kvam, 1971). Furthermore calcium

metabolism in rats seems to be controlled by intestinal absorption rather than the bone itself (Ren et al., 2004). Differences have also been reported in the structure and arrangement of periodontal fibers as well as supracrestal fibers. Moreover rat PDL has been found to be devoid of any elastic fibers (Reitan and Kvam, 1971). Other differences include the fact that rat molars drift distally compared to mesially drifting human molars in addition to the fact they have continuously erupting incisors (Thilander et al., 2005). Lastly, although the principal mechanism is the same, tissue changes incident to orthodontic loading seem to be a lot faster in rats than in humans.

Despite the many differences between rats and humans, using the rat model offers several advantages which makes it the most popular animal model used to study orthodontic tooth movement (Ren et al., 2004).

- Firstly rats are relatively cheap and easy to house for long periods of time which facilitates the use of large samples compared to various difficulties in larger animals such as dogs or monkeys (Ren et al., 2004).
- Secondly it is easier to prepare histological samples from rat material than other larger animals (Ren et al., 2004).
- Using rats or also animals in general offers the possibility to control the environmental influences as well as the diet which makes isolating the variables of interest to the study achievable. This is almost impossible to control in humans. This becomes even more important when the testing of drugs and pharmacologic agents is required.

- Fourthly antibodies that are required for specific immune-histochemistry are almost exclusively available for rats and mice (Ren et al., 2004).
- Lastly transgenic strains and specific gene-knockout animals are only available from small rodents, most likely mice, but also some rat strains as well (Al-Qawasmi et al., 2006).

7.4 Methods used to measure tooth movement

Tooth movement can be evaluated in various ways; one is to measure the distance travelled over a certain period of time. Several methods have been applied in the literature to measure tooth movement in rats with every method having certain limitations. Some studies that have only loaded the first molar with a mesially directed force, attempted to measure the tooth movement by measuring the gap between the first and second molars. One study employed leaf gauges to estimate that distance (King and Fischlschweiger, 1982). This method has inherent problems such as the difficulty in getting exact measurements especially if the distance lies between two gauges. It should also be noted that orthodontically loaded teeth are relatively mobile and so placing the leaf gauge may displace the teeth leading to an overestimation of tooth movement. Another method reported was measuring the distance the first molar moved on standardized lateral head films of the rats. The disadvantages in this method are firstly; the need for a standardized radiography method and availability of an x-ray machine.

Secondly’ it requires prolonging the period of anesthesia for the animals. The other problem is more fundamental and relates to the reliability and reproducibility of the images. Superimpositions of the other structures and of the teeth from the contralateral side are likely to be a problem. Lastly the x-ray beam is not perpendicular to the contact area thus making the estimated distance not necessarily accurate (figure 18) In addition magnification and image distortion are also a problem.

Several studies have reported the use of digital calipers to measure the tooth movement as the decrease in distance between the mesial surface of the first maxillary molar and the distal surface of the ipsilateral incisor (Ren et al., 2004). Although relatively accurate there are several limitations to this method. The angulations at which the calipers are held can influence the measurement which is made more difficult by the small size and limited access within the rats’ mouth. Furthermore unless they are prevented, rat incisors are continuously erupting and this can change the distance to the first molars (Ren et al., 2004).

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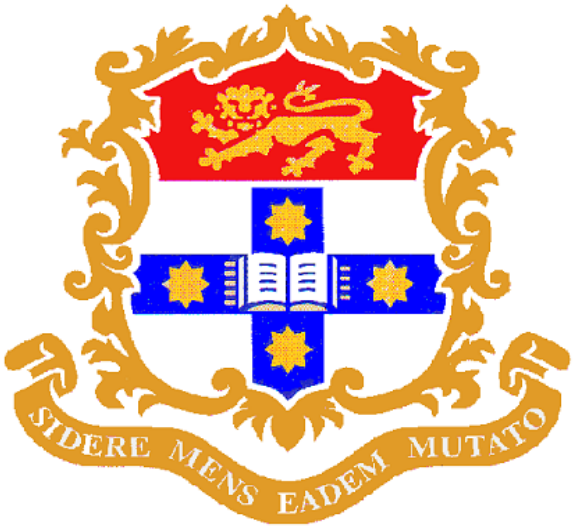
9 Manuscript



A study of the influence of combined Glucosamine Sulfate and Chondroitin Sulfate systemic supplements on root resorption and tooth movement in rats

A condensed version of this manuscript is to be submitted for publication

A study of the influence of combined Glucosamine Sulfate and Chondroitin Sulfate systemic supplements on root resorption and tooth movement in rats

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9.1 Abstract

Root resorption as a side effect of orthodontic force is problem that has plagued orthodontists for a long time. Orthodontic tooth movement is considered to be an inflammatory process and is usually associated with some degree of root resorption. Glucoseamine Sulfate and Chondroitin Sulfate are now widely used as anti inflammatory and chondroprotective agents for osteoarthritis. They act by reducing the expression of the same inflammatory agents responsible for the tooth movement and root resorption.

Aim: The aim of the study was to examine effect of glucosamine and chondroitin sulphate on root resorption and tooth movement in response to heavy and light orthodontic forces.

Materials and methods: 80 Wistar rats were divided into 4 groups of 20, two experimental and two control groups. Group 1 received GS and CS in their diet and were assigned to appliances with light continuous forces of 10 cN. Group 2**:** received GS and CS in their diet and assigned to appliances with heavy continuous forces of 150 cN. Group 3**:** received normal diet without any drugs and were assigned to appliances with light continuous forces of 10cN. Group 4: received normal diet without any drugs and were assigned to appliances with heavy continuous forces of 150 cN. Forces were applied using NiTi coil springs (Sentalloy GAC, New York, USA) the maxillary first molar of one side to the maxillary incisors. The animals were fed GS and CS for 2 weeks prior two appliance insertion two insure adequate plasma levels. Appliances were activated for a 2 week period after which the animals were sacrificed. All samples were scanned using

SkyScan 1072 Micro CT and analysed with VGstudiomax 1.2v software. Tooth movement was assessed by analysis of the distance between the first and second molars. Total volume of root resorption crates was estimated.

Results: Root resorption craters were found to be concentrated at the mesio-cervical portion of the roots. The results showed that GS and CS had the effect to reduce root resorption but the result was only marginally significant (p=0.077) while they had no effect on tooth movement (p=0.60). There was no difference in the amount of root resorption between the heavy and light force groups (p=0.85). It was also found that heavy forces produced almost double the amount of tooth movement than light forces (p=0.012).

Conclusion: The findings of this study indicate that systemic administration of GS and CS may reduce root resorption incident to orthodontic tooth movement while not affecting the rate of tooth movement although further research is required to clarify the mechanism of action of these supplements on the periodontium and whether they could potentially be used during the course of orthodontic treatment.

Key words: Glucosamine sulfate, Chondroitin sulfate, Root resorption, Orthodontic tooth movement, 3D micro CT, Volumetric analysis, Wistar Rats

9.2 Introduction and review of the literature

Orthodontic tooth movement and root resorption have been extensively studied in orthodontic literature [1, 2]. Root resorption is an undesirable and still considered an unavoidable side effect of orthodontic treatment [3, 4]. It is well documented that inflammatory mediators, neurotransmitters and growth factors as well as numerous other cytokines such as IL-1 play a vital role in orthodontic tooth movement as well as its side effects including pain and root resorption [1, 5-7]. Thus root resorption associated with orthodontic treatment was termed orthodontically induced inflammatory root resorption OIIRR [3, 4]. Any pharmacologic agents and nutritional supplements consumed by the patient can reach the periodontal tissues through the circulation and thus interact and influence the cells and molecules altering their response to orthodontic forces [8]. These agents may have the effect to potentiate or inhibit tooth movement as well as exacerbate or reduce root resorption. The effect of several medications on tooth movement and root resorption has been extensively studied [8].

Prostaglandins (PGs) are unique in the fact they can stimulate both bone resorption and formation and so have been found to play an important role in the tooth movement and root resorption process [1]. Local injection of prostaglandins has been found to accelerate tooth movement [9-11] but it has also been found to increase the amount of root resorption [12, 13]. In addition injections of prostaglandins are very painful [14].

On the other hand non-steroidal anti-inflammatory drugs (NSAIDs) inhibit prostaglandin secretion by inhibition of the cyclooxygenase enzyme (COX). Conventional NSAIDs such as ibuprofen and aspirin have been found to reduce tooth movement by reducing the number of osteoclasts due to the inhibition of prostaglandin secretion [15]. A study by *Villa et al* found that the NSAID nabumetone significantly reduced the amount of root resorption while not significantly impeding tooth movement in human premolars but the exact mechanism is not understood. Selective COX-2 inhibitors have the advantage of relieving pain and having a strong anti-inflammatory effect while avoiding gastric irritation caused by conventional NSAIDs. Studies have demonstrated that different types of COX-2 inhibitors can affect tooth movement and root resorption differently. In a study by *de Carlos et al* it was demonstrated that while rofecoxib inhibited tooth movement, celecoxib and parecoxib did not, with celecoxib showing the least effects on tooth movement [16, 17]. Furthermore one study suggested that celecoxib (Celebrex) may offer some protection against orthodontically induced root resorption in rats with minimal inhibition of tooth movement but further research is needed to confirm those results [18]. NSAIDs are usually prescribed in orthodontics to control pain associated with initial activation of the appliances, *not* to prevent or reduce root resorption as this would require long term use over the course of the treatment with the risk of the side effects of long term use of NSAIDs being gastric irritation and potential ulceration.

The effects of corticosteroids have also been studied. Several studies have shown that corticosteroids may slow down orthodontic tooth movement [19, 20]. Their effect on root resorption on the other hand has been somewhat controversial. Relatively large doses

(15mg/kg) used acutely with orthodontic forces on rabbits showed significantly more root resorption than controls [19] while in another study the opposite was demonstrated with small doses (1mg/kg) [21].

The effect of acute and chronic corticosteroid administration has been compared by *Verna et al* [22] on rats. They found that acute administration of corticosteroids increased the risk of root resorption while chronic administration showed no significant difference. This may be due to the fact that corticosteroids may reduce or inhibit osteoblastic activity by increasing the blastic cycle of the osteoblast thus allowing more osteoid to be present which cannot be resorbed by osteoclasts, while enhancing or not changing clastic activity. This would favour more root resorption. The authors then advised that in cases where a patient starts an acute course of corticosteroids it may be advisable to go into a passive phase in the orthodontic treatment or if active treatment is continued to closely monitor for root resorption with periodic radiographs.

Bisphosphonates are well known potent inhibitors of bone resorption that are used in many bone and metabolic disorders [8]. Several studies have examined the possible effects of bisphosphonate administration on orthodontic tooth movement as well as root resorption [23-27]. Most studies have found that bisphosphonates whether used systemically or applied topically tend to inhibit tooth movement as well as reduce root resorption. Although the use of bisphosphonates to prevent orthodontically induced root resorption is still unlikely due to their inhibition of tooth movement they may prove

useful in the prevention of inflammatory root resorption incident to tooth replantation following trauma when orthodontic movement is not necessary.

Mavragani et al [28] found that doxycycline given in low sub-antimicrobial doses did not affect the rate of tooth movement but had an inhibitory effect on root resorption with the experimental group showing less root resorption than the controls. It is worth mentioning that several studies have reported gastro intestinal disturbances as well as the development of tetracycline resistant microbial strains [29, 30] as potential side effects which makes this medication a questionable option for wide use as a preventive measure to OIIRR [31].

The effect of systemic fluoride administration on root resorption has also been studied [32]. Although there is a tendency towards less resorption with high fluoride intake, the levels used in the study would be considered toxic for humans. Nevertheless this may indicate that patients with history of fluorosis may show less root resorption and a further study into this is currently underway.

The effects of thyroid hormone administration have also been studied. *Shirazi et al* [33] have demonstrated that increased levels of thyroid hormone can significantly increase the rate of tooth movement. It is thought that it increases the efficiency of bone remodeling. *Poumpros et al* [34] studied the effects of low dose L-thyroxin administration on OIIRR in rats. They found the L-thyroxin group exhibited 50% less resorption than the control group and based on their findings they prescribed low dose thyroid hormone replacement

for three patients who were thought to be “high risk” for root resorption [35]. However long term thyroid hormone treatment to accelerate tooth movement and/or prevent root resorption, except for the most severe cases, is difficult to justify. Long term thyroid hormone treatment may have serious systemic side effect that may outweigh the benefits of orthodontic treatment [36, 37].

Recently nutritional supplements glucosamine (GS) and chondroitin sulfate (CS) have been introduced in the management of OA and related symptoms [38, 39]. Osteoarthritis (OA) is a chronic disease which is characterized by irreversible damage to joint structures. This includes loss of articular cartilage, formation of osteophytes, alterations in the subchondral bone and synovial inflammation [40]. GS and CS have been reported to relieve pain associated with osteoarthritis as well as aid in the reduction of cartilage and joint degeneration but the exact mechanism of action remains unresolved [38, 39]. They belong to a category of compounds that have a slow acting symptomatic effect in OA and so were termed “symptomatic slow-acting drugs for OA (SySADOA) [40]. Most of the compounds suggested as SySADOA are naturally occurring in the body and articular tissues. Glucosamine supplements are mostly derived from bovine or shellfish chitin and recently production from a vegetarian source has also been introduced [41]. CS can be obtained from animal or marine cartilage such as bovine, porcine or shark cartilage with CS from bovine trachea cartilage being the most widely used in clinical trials [42].

GS and CS are termed nutraceuticals [43] in most countries which makes them available over the counter as nutritional supplements. This is mainly due to their long term well documented safety profiles. A recent review by *Hathcock et al* concluded that no adverse effects of GS and CS could be identified in the literature [44]. Although some concerns were raised with glucosamine having a diabetogenic effect, animal and human trials have not supported this view [38, 39, 45].

GS and CS are naturally occurring in the body. Glucosamine is a monosaccharide which is part of a larger group of molecules glycosaminoglycan GAG that is incorporated into the cartilage proteoglycans [46]. CS is a major component of many of the bodies' connective tissues including cartilage, bone, tendons, ligaments and skin [40]. It is a sulfated glycosaminoglycan that is composed of a long unbranched polysaccharide chain with the repeating disaccharide structure of N-acetylgalactosamine and glucuronic acid. N-acetylgalactosamines are mostly sulfated usually in position 4 and 6 (CS4 and CS6) making it a strongly charged polyanion. CS is believed to play a major role in imparting the articular cartilages' ability to resist stresses during various loading conditions by providing it with resilience and elasticity [40].

Several mechanisms of action of these agents in OA have been suggested. Originally it was thought that the compounds only provided the building blocks for cartilage and as an exogenous source for cartilage matrix components [47]. Research has also demonstrated that they can normalize cartilage metabolism as well as reduce cartilage degeneration

[48]. Invitro studies have shown that glucosamine can stimulate the synthesis of cartilage glycosaminoglycans (GAGs) and proteoglycans [49].

Further more GS and CS have demonstrated anti-inflammatory properties with reduction and down-regulation of several inflammatory mediators such as PGE2, NO and IL-1 [50-53]. *Chan et al* reported that physiologically relevant concentrations of GS and CS regulate gene expression and synthesis of nitric oxide (NO) and prostaglandin (PGE_2) this may provide an explanation for their anti-inflammatory properties [54].

GS and CS have also been shown to reduce the effects of matrix degrading enzymes such as proteases. Matrix metalloproteinases (MMPs) are a particular group of enzymes that play an important role in the remodeling of cartilage, bone and other calcified tissues during physiological as well as pathologic remodeling [55]. CS has been found to decrease the effects of MMPs by down regulating their synthesis as well as reducing their activity thus accounting for their connective tissue protective properties [51, 56-58].

CS has also been shown to decrease apoptosis of chondrocytes [59] which has been found to be increased in OA cartilage. Furthermore the effect of CS on bone resorption factors has recently been studied [60] on human OA sub-chondral osteoblasts after the stimulation with vitamin D3. The results showed that CS up-regulated the ratio of OPG:RANKL i.e. it up-regulated OPG and also down-regulated RANKL. Considering that in OA abnormal osteoblasts increase the expression of RANKL and increase bone resorption; CS would have a positive effect on bone protection [40].

Since inflammation is an integral part of the tooth movement and the root resorption mechanism and matrix degradation with bone/cementum resorption are intimately related, GS and CS may have a role to play in modifying those mechanisms thus influencing tooth movement and root resorption. The effect of GS and CS administration on tooth movement and root resorption has not yet been studied. Due to the increasing number of adults using GS and CS and the rising number of adults seeking orthodontic treatment it is important to investigate their potential effects on tooth movement and root resorption.

9.3 Aim

The aim of the study is to evaluate the effect of systemic administration of the nutraceuticals glucosamine and chondroitin sulfate, on the rate of tooth movement and root resorption when heavy and light orthodontic forces are applied to rat molars.

9.4 Materials and methods

9.4.1 Study Design

Ethical approval was obtained from the University of New South Wales Animal Care & Ethics Committee (ACEC Number: 07/32A) in May 2007. The sample was composed of 80 laboratory Wistar rats, eleven weeks of age. This study used a sample of 11 week old

Wistar rats to ensure complete development of the root dentine, cementum, PDL and alveolar bone which are completed by 8 weeks of age [61]. The experimental and control animals were allotted to groups of approximately equal weight in order to minimize any unintentional bias of assigning heavier animals to either group. The animals were divided into 4 groups, two experimental and two control groups (Table 1).

Group 1: received GS and CS in their diet and were assigned to appliances with light continuous forces.

Group 2: received GS and CS in their diet and assigned to appliances with heavy continuous forces.

Group 3: received normal diet without any drugs and were assigned to appliances with light continuous forces.

Group 4: received normal diet without any dugs and were assigned to appliances with heavy continuous forces.

Housing: –The animals were placed in boxes with two animals in each box, so that they were not alone but had adequate space for to hide and play. The animals were monitored daily and staff interacted with them through handling and normal interaction.

9.4.2 Feeding and GS&CS doses

Experimental and control animals were given the same diet of Nutrigel (Troy Laboratories PTY LTD, NSW, Australia) mixed with rat chow with the same frequency and had free access to water throughout the whole experimental period. Nutrigel is a commercial veterinary nutritional supplement (the Australian equivalent of Nutri-cal used in this study). It is a highly palatable oral supplement used in veterinary medicine to provide either partial or full nutritional support for mammals. In addition the experimental rats received combination of GS and CS which was mixed with the Nutrigel and rat chow on a daily basis. The control rats did not receive any additional supplementation.

The rats were fed the diet for two weeks to give a loading period for the GS and CS to reach adequate plasma levels [62] before the placement of the orthodontic appliances.

The supplementation was given orally as research has shown that this form of supplementation is well absorbed via the oral route [47, 63]. This feeding technique avoids the inherent stress associated with restraint and gastric tubing.

Dosage: The calculation of the dose was based upon the weight of the rat. The powder form of glucosamine and chondroitin sulphate was homogenized using a power mixer, and combined with the commercial veterinary nutritional supplement Nutrigel. Rats receiving no supplementation received 5.3ml of Nutrigel mixed with rat chow every 24 hrs. Rats in the supplementation group received 5.3ml of Nutrigel homogenized with a

dosage of 1.4-1.6 g of glucosamine/ kg (250 mg of glucosamine/rat), and 1.15-1.3g of low molecular weight sodium chondroitin sulfate/kg (200mg of sodium chondroitin sulfate/rat) mixed with rat chow every 24 hours. The dosing in this study was based on the study by *Echard et al* [45] which indicated that the therapeutic dose effective in rats is in the range of 10-20 times the human dose of 1500mg/day for a 70kg human for glucosamine sulphate, which would mean a concentration of 0.5%w/w of Glucosamine. Similarly it is advised that the chondroitin sulphate rat dosage compared to the human dose would be in the range of 3-7 times, which would hold true for CS (0.4%w/w). This corresponds to an average recommended daily dose for humans of 80% that of glucosamine, 1200mg/day.

9.4.3 Orthodontic force application

Orthodontic forces were assigned randomly to either the right or the left side of the mouth. Light continuous forces were applied using a 3mm Sentalloy closed coil spring (10 cN, wire diameter 0.22 mm, eyelet diameter 0.56 mm GAC, New York, USA) and Heavy continuous force were applied using a 3mm Sentalloy closed coil spring (150 cN, wire diameter 0.22 mm, eyelet diameter 0.56 mm GAC, New York, USA). The NiTi coils were tied to the first maxillary molar using 0.08 stainless steel ligature wire and stretched over the 8-10mm distance to the maxillary incisors where it was tied with an 0.08 SS ligature in the shape of a figure 8 around the maxillary incisors. The ligature wires were then secured to the teeth by bonding using a self etching primer (Transbond™ Plus Self Etching Primer 3M Unitek) and composite resin (Transbond XT™ Light Cure Adhesive

3M Unitek) (Figure 1 and Figure 2). The appliance design is similar to that described by *Brudvik and Rygh* [12] which was also used by others [18, 22].

The coils were stretched to a distance of 8 mm on average which is more than double the length of the coil to ensure continuous and constant force levels as described by *Miura et al* [64].

Anesthesia – The animals were induced and maintained using isoflurane 2% and oxygen 2% inhalation during appliance placement. They were constantly monitored for changes in vital signs during surgery. Once the animals were operated on (i.e. attachment of the coil springs), they were allowed to recover. They were given temgesic pre-operatively for pain relief at a dose of 0.01mg/kg and Cephazolin sodium, an antibiotic to prevent infection. Animals were monitored carefully at regular intervals throughout the day until the end of the study. Staff who were feeding the animals and changing the bedding checked for any discomfort or abnormalities. Animals had free access to food and water through out the experiment. The rats were monitored daily for signs of distress and pain based on behavioral changes, eating patterns and were monitored daily for their ability to feed and groom. They were also monitored for signs of diarrhea and vomiting. A period of orthodontic force application of 2 weeks was chosen as repair processes were found to dominate after that [65].

9.4.4 Sample preparation

After 2 weeks from appliance placement the animals were sacrificed and then decapitated. The skin and mucous membrane were dissected and the maxilla separated from the skull with an oscillating surgical saw. The maxillary buccal segment, at the site of force application, was then separated from the maxilla using a fine diamond saw. The samples were then stored in formalin.

9.4.5 Scanning and data collection

Skyscan 1172 micro CT was used to scan the maxillary molar segment. Skyscan 1172 micro CT is a compact, desktop x-ray system used for the non destructive three dimensional imaging of small samples with a resolution of up to 2 microns (Figure 3). It is considered a miniature version of conventional medical computed tomography (CT). In addition to the size and resolution differences another difference between microCT and conventional medical CT is that in conventional CT the beam and the sensor rotate around the subject while in microCT the specimen is placed on a rotating platform while the x-ray source and sensor are fixed (Figure 4). A detailed description of the scanning method and image reconstruction used has been described by *Foo et al* [32].

9.4.5.1 Scanner settings

The samples were scanned at a resolution of 6 microns with an X-ray beam of 100 Kv and 100mA with no filters. Throughout the scanning procedure, the samples were rotated 360 degrees and a scanning period of 2 seconds per degree of rotation with a frame averaging of 2. The average scanning time per sample was around 1 hour and 15 minutes.

The raw data collected was then reconstructed to provide a series of axial pictures (Fig. 4) in cross section. The images were reconstructed using Reconstruction Program=NRecon version 1.4.2. with around a thousand cross sections collected per sample. The time taken for the reconstruction was approximately 6 hours per sample. Through this procedure, the raw data was converted to 8 bit, Bit Mapped Picture files, with a resolution of 1024x1024 pixels. The images were reconstructed at a smoothing of 2, ring artefact reduction of 5 and beam hardening reduction of 50%. These images could then be imported into any 3-D visualization and analysis software, which in this study was VGStudioMax version 1.2.

9.4.5.2 VGStudioMax version 1.2

This was the software package used to reconstruct the axial data collected into a viewable three dimensional reconstruction of the scanned sample. The scanned images included both the maxillary molars and the surrounding bone structure (Figure 5). Using the software, the bone could be removed from the images, leaving the tooth for further

analysis, i.e. software extraction (Figure 6). This eliminates the risk of damaging the fragile rat molar root with manual extraction. The three dimensional image of the tooth could then be manipulated (e.g. rotated) to allow for visualisation of the root surface and examination of root resorption craters (Figure 7). Once the resorption craters were located, they can be further isolated using software.

9.4.5.3 Analysis of root resorption crater volumes

Root resorption craters could be identified on all roots of the maxillary molar but were most clearly and consistently identifiable on the largest of the roots, the mesial root. To ensure consistent recording of the location of the root resorption craters it was decided that only the mesial root of the first maxillary molar would be used. Additionally the apical section of the molar roots was found to be too porous, due to the presence of numerous accessory canals and surface irregularities, making it difficult to differentiate between normal tooth anatomy and root resorption craters. However there was a distinct demarcation of the porous section of the molar root, which could be delineated accurately to within a few axial sections. It was therefore decided that root resorption craters present on the mesial root of the first maxillary molar between the cemento-enamel junction and the start of the porous apex would be measured (Figure 8).

The analysis of root resorption crater volumes was done digitally using the VGstudiomax 1.2v package. For the purposes of quantifying the volume of the root resorption craters, the images were viewed in axial sections through the root. The resorption craters were

identified in the axial plane. Then a ‘mask’ was created for the volumetric analysis of the crater images. The procedure was as follows (Figure 9,Figure 10 and Figure 11). Firstly the segment of the mesial molar root between the CEJ and the porous apex was defined. Then a map, which describes the coordinates and location of the root resorption craters, was created.

The third step is then to create a digital ‘mask’ for each crater. This is done as follows: The axial sections at which the crater began and ended were located. The VGstudiosMax segmentation tool is then used to draw an outline of the crater. The outline was drawn so that it followed the internal contours of the crater while the external border of the crater was drawn as an estimation of the continuation in convexity of the root surface. The continuation in convexity was estimated by connecting the 2 points at the edge of the break in convexity.

The mask was then propagated through the axial sections of the tooth for no more than 6 slices. The propagation of the mask was discontinued if the limits of the crater were reached or if the mask no longer followed the outline of the crater. If the mask ceased to resemble the crater a new outline was drawn.

After the masks were created for all the craters they were all summed into a single segmentation (Figure 12). VG StudioMax software then calculated the volume of the segmented mask which was recorded as the total volume of the root resorption for that root.

9.4.5.4 Tooth movement measurement

Tooth movement was measured digitally utilizing the 3D microCT images to measure the distance between the first and second molars. The measurement was done using a special software tool that is designed to identify and measure the closest distance between two parallel or nearly parallel surfaces or lines (Figure 13). This can overcome a lot of the variability involved in manual human measurement using calipers or gauges. This measurement was done with the assumption that the teeth were in tight contact prior to appliance placement.

Since the software tool can only be used in two dimensional images, tooth movement distance was measured in the axial sections and then in the sagittal sections then an average of the two measurements was taken to overcome any slight variation.

The measurement was taken in a stepwise fashion. Firstly the observer scrolled down through the axial sections of the contact area between the 1st and second molars and visually identified the axial section that appeared to show the shortest distance between the two surfaces. This axial section was used as a starting point. The tool was then employed to identify the line of shortest distance between the distal surface of the first molar contact area to the second molar contact area. This identified the shortest distance between the two surfaces in the axial plane i.e. in the buccolingual dimension. The sagittal sections (bucco-lingual sections) at which this line of shortest distance falls were

then viewed on the screen. The same software tool was then used to identify the narrowest point between the heights of contour of the proximal surfaces of the first and second molars (Figure 14)`1. This identified the shortest distance between the two surfaces in the occluso-gingival dimension. Lastly this line was then used as reference to locate the axial section at which this shortest distance occurs and then the same software tool was used to measure the shortest distance between the two surfaces in the axial plane again but this time using the sagittal measurement to locate the correct axial slice to measure (Figure 15). The sagittal measurement and the last axial measurement were then averaged, if they were not identical, which could have been due to slight variation in the angle at which the sample was originally scanned.

9.4.6 Statistical Analysis

Data collection was performed by the same operator. Statistical analysis was performed by SPSS version 14 for Windows (SPSS Inc., Chicago, Illinois, USA). A Univariate Analysis of Variance was performed to test the effect of the drug and the force magnitude as covariates with total volume of the root resorption craters as the dependant variable. Another analysis of variance was performed to test the effect of the drug and the force magnitude as covariates and the total amount of tooth movement as thee dependant variable. To test the effect of the weight change of the animals both analysis were repeated with the weight of the animals as a covariate. Bonferroni adjustments were done for the multiple comparisons.

An error study was conducted where the same observer measured the root resorption volume and tooth movement on 10 samples on two separate occasions. Analyses of the error of the method revealed that the coefficient of variation for root resorption volume was is 7.9%, while the equivalent results for the tooth movement measurements were between 1% for the axial measurement and 3% for the sagittal measurement.

9.5 Results

9.5.1 Weight and health of the animals

All rats in the control and experimental groups gained weight during the period of the experiment but rats in the GS and CS groups gained less weight than the controls. It was felt that appliance breakage may have interfered with the animals' ability to feed and so the animals with appliance failure were eliminated from the analysis. It was found that the average weights of the rats at the start of the of the experiment for experimental groups 1 and 2 and for control groups 3 and 4 were 206 ± 13g and 207 ± 17g respectively the difference was not found to be statistically significant(Table 2) and (Table 3). On the other hand the weight of the animals at sacrifice was found to be 223.5 ± 17g and 245 ± 16g for the experimental groups 1 and 2 and the controls group 3 and 4 respectively (Table 4). This was found to be statistically significant ($p<0.001$).

2 animals from the experimental group were lost due to anaesthetic death.

9.5.2 Appliance breakage

Twenty two of the light force appliances failed, seventeen of which were due to fracture of the coil spring and five due to various other reasons such as ligature wire failure and bond failure on the incisors. Thirteen of the failed appliances were in the experimental group and eight in the control group. Eight of the heavy force appliances failed; three due to fracture of the coil spring and three due to composite fracture on the incisors and two due breakages at the molar. All animals with failed appliances were eliminated from the analysis. After sample attrition the available samples for analysis were as follows six in Group 1, fourteen in Group 2, twelve in Group 3 and seventeen in Group 4.

9.5.3 Root resorption

From observations on the three dimensional reconstructions root resorption craters could be clearly identified on the root surfaces and there appeared to be a pattern to their distribution. Root resorption lesions were evident in the coronal cervical part of the mesial surface of the roots which is consistent with free tipping movement (Figure 8). It was difficult to identify craters in the apical part of the roots due to the porosity and surface irregularity with the presence of numerous accessory canals.

9.5.4 Root resorption volume

The mean crater volume in the molars of rats that had GS and CS was 50% less than that found in the control samples. A Univariate Analysis of Variance comparing the total volume of craters within the four groups (Table 5), showed that GS and CS demonstrated a statistically significant reduction in the total volume of root resorption (p=0.007) (Table 6) and (Table 7). Bonferroni adjustments were done for the multiple comparisons. On the other hand the force magnitude variable did not have a statistically significant effect on the root resorption volume between the light and heavy force groups (Figure 16). Considering that the weight of the experimental groups was lower than that of the controls at sacrifice the Analysis of Variance was redone to include the weight of the animals as a covariate. The results showed that GS and CS still had the effect to reduce root resorption but the result was only marginally significant (p=0.077) while the effect of force magnitude remained statistically insignificant (p=0.85) (Table 11), (Table 12) and (Table 13).

9.5.5 Amount of tooth movement

A Univariate Analysis of Variance ANOVA comparing the amount of tooth movement within the four groups showed that GS and CS demonstrated no significant effect on tooth movement while force magnitude showed a significant effect on tooth movement (p=.007) (Table 8),(Table 9) and (Table 10). Heavy forces produced almost double the

amount of tooth movement calculated in the light force group. Bonferroni adjustments were done for the multiple comparisons (Figure 17). When the results were examined with ANOVA using the weight of the animals as a covariate the results remained the same. GS and CS demonstrated no statistically significant effect on tooth movement ($p=0.60$). On the other hand force magnitude displayed a statistically significant effect on tooth movement ($p=0.012$) with heavy forces producing almost double the amount of tooth movement (Table 14),(Table 15) and (Table 16).

9.5.6 Error of the method

An error study was conducted where the same observer measured the root resorption volume and tooth movement on 10 samples on two separate occasions. Analyses of the error of the method revealed that the coefficient of variation for root resorption volume was is 7.9%, while the equivalent results for the tooth movement measurements were between 1% for the axial measurement and 3% for the sagittal measurement (Table 17).

9.6 Discussion

9.6.1 Methodology

The aim of this study was to investigate the effects of GS and CS administration on orthodontically induced inflammatory root resorption and orthodontic tooth movement. The study was conducted on an animal model to allow the control of dietary and environmental factors and to standardize the dosage of the drugs. Eleven week old Wistar rats were selected to ensure complete development of the root dentine, cementum, PDL and alveolar bone which are completed by 8 weeks of age [61]. Wistar rats have been commonly used in tooth movement [66] and root resorption experiments [18, 22, 32].

Orthodontic forces were applied using super-elastic NiTi coil springs which were stretched from the maxillary first molar to the maxillary incisors. This appliance design was described by *Brudvik and Rygh* [12] and was used for numerous root resorption as well as tooth movement experiments [18, 22, 67]. Two force levels were used 10cN for the light force groups and 150 cN for the heavy force groups. According to a review by *Ren et al* on the use of the rat model for tooth movement the lightest forces reported in the literature were 20 cN [66]. Heavy forces of greater than 60-80 cN are reported to produce substantial cemental cratering [18, 22, 68] and thus 150 cN were used in the heavy force group to ensure root resorption would take place. A period of 2 weeks of orthodontic loading was selected to allow recording of the volume of root resorption without it being masked by repair. It was demonstrated that by 1 week of loading root

resorption craters were already identifiable and that after 2 weeks the repair process was found to be predominant [65]. There was a two week period of drug administration prior appliance placement to allow sufficient plasma levels of GS and CS in order to produce a pharmacologic effect. GS and CS are considered to be slow acting drugs in the treatment of OA (SySADOA). Their maximal effect is only attained after several months of treatment in humans [69] and they also have a carryover effect that persists after treatment is stopped [40]. Nevertheless a period of two weeks was found to be sufficient to produce sufficient effect in rats [62].

9.6.2 Tooth movement measurement

This study has presented a novel approach to measuring tooth movement in rats. Using three dimensional microCT images of the buccal segments the distance between the first and second molars could be accurately assessed using a software tool thus eliminating many of the errors involved with manual human measurements. This measurement has been found to be accurate and reproducible to within 1-3%. The method eliminates many of the limitations of previously reported methods. Several studies have reported the use of digital calipers to measure the tooth movement as the decrease in distance between the mesial surface of the first maxillary molar and the distal surface of the ipsilateral incisor [66]. Although relatively accurate there are several limitations to this method. The angulations at which the calipers are held can influence the measurement which is made more difficult by the small size and limited access within the rats' mouth. Furthermore unless they are prevented, rat incisors are continuously erupting and this can change the

distance to the first molars [66]. Compared to measurements on lateral cephalograms the method used in this study offers many advantages. Firstly superimposition of structures from the other side of the skull can make accurate assessment of the 'gap' between the first and second molar difficult. Furthermore beam angulations in relation to the tooth movement can also mask the true amount of tooth movement (Figure 18). In addition image magnification and distortion is also a problem.

9.6.3 Root resorption measurement

This study used three dimensional microCT images to quantify the root resorption volume. Several methods have been reported in the literature with regards to quantification of root resorption in experimental research. Two dimensional images utilizing serial histological sections studied by light microscopy may shed light on the cellular and tissue level interactions and changes that play a role in root resorption. Their use in quantifying root resorption has been questioned [70]. Scanning electron microscopy has also been used with several limitations [70]. Firstly the root surfaces are curved which makes a straight on view of the crater not easy to achieve this also introduces a parallax error. The two dimensional images of the craters are also obtained on micrographs which are then pieced together and then the craters are measured with a digitizer. This may also introduce an error especially for craters at the edges of the micrographs. Due to the two dimensional nature of this imaging technique the true extent of root resorption craters may have not been adequately represented [70].

Three dimensional imaging using microCT offers a non destructive way to visualize tooth roots and volumetrically quantify root resorption giving a more accurate representation of the extent of resorption. This tool has been used with success for human premolars [71, 72] as well as on rat molars [32] although some problems were faced in this study with regards to the anatomy of the rat molar roots. The apical portion of the molars is very porous due to the presence of a multitude of accessory canals and the surface of this part of the root is also very irregular. This makes it difficult to isolate root resorption craters for volumetric assessment. For this reason it was elected to only quantify craters in the middle and cervical part of root (Figure 8). Nevertheless due to the tipping nature of the tooth movement applied in this study root resorption craters were concentrated at the mesio-cervical portion of the roots which is the area that has been analyzed. This is also consistent with other studies [22, 67] that found that mesio-cervical portion of the roots showed the greatest changes when tipping movement was used.

Assessment of the error of the volumetric measurements found the method to be reasonably accurate and reproducible with the coefficient of variation for root resorption volume being 7.9% (Table 17).

9.6.4 Effect of heavy versus light force on orthodontic tooth movement

A statistically significant difference could be identified with regards to the amount of tooth movement between the heavy and the light force groups (Figure 17). The heavy

force groups with 150cN showed almost double the amount of tooth movement when compared to the light force groups with 10cN over the two week loading period. Although this may indicate that heavy forces move teeth faster than light forces, it should remembered that the loading period of two weeks used in this was relatively short to make such estimates. A recent study by *Gonzales et al* found that tooth movement over the first 14 days of force application was not sensitive to force magnitude while at 28 days the 10cN force group demonstrated more tooth movement than 100cN group [73]. It may be that if the forces were left for a longer period of time the light force group would have caught up with the heavy force group. It should also be noted that individual variation in the response to orthodontic force magnitude was large with some animals in the light force groups showing more tooth movement than animals in the heavy force groups. This is in agreement with several studies who found large individual variation in the response to force magnitude [74-76].

9.6.5 Effect of heavy versus light forces on root resorption

The results of this study indicate that there was no difference between the application of a continuous force of 10 cN and 150 cN on the amount of root resorption on the rat first molar (Figure 16). These findings seem contradictory to several other studies that found more root resorption with heavy forces than with light forces when root resorption was volumetrically quantified using similar methodology [72, 77, 78]. Nevertheless all of these studies used human subjects with the forces used being in the range of 25 g for the light force groups and 225 g for the heavy force groups. Root resorption has been

identified to take place at the “hyalinized zone” in relation to tissue damage with those areas of the root being “marked” by the damage and resorption taking place. For this reason *Reitan* suggested using light forces in orthodontic treatment to allow the stimulation of a cellular response while minimizing the side effects in the form of root resorption [79]. *Vardimon et al* [80] explained the determinants of root resorption in response to loading with the magnitude of force being a significant determinant but in combination with the duration of force. Rat molars are very small compared to human teeth (approximately 50 times smaller) [66]. Pressure on the PDL with orthodontic loading depends on the magnitude of the force and surface area of the PDL loaded. This means that the light force of 10 cN applied on the rat molar would be equivalent to 500 cN on a human molar which places the force in the high force category of the above mentioned studies. This can explain why similar amounts of root resorption were observed in the light and heavy force groups in this study. The force in the 10 cN group was still high enough to cause similar hyalinization to that caused by 150 cN therefore the biologic response was the same amount of root resorption for both forces. It is practically very difficult to produce forces that are light enough to simulate a 25 cN force on human teeth on the rat model. *Ren et al* used a 10 cN coil applied to all 3 maxillary molars together [66]. This may reduce the force level to that equivalent to 170 cN in humans (10/3x50) which would still be considered relatively heavy. Furthermore coils that deliver this force magnitude are very fragile and susceptible to damage even when the rats were kept on a relatively soft diet. Seventeen out of forty appliances broke in the light force group due to coil failure while the heavy coils had a better survival rate. Another factor to consider is the possibility that occlusal trauma from the composite resin

used on the molars to secure the appliances may have contributed to the root resorption. Few case reports have linked occlusal trauma to severe root resorption [81-83] but it is very difficult to assess what effect this could have had on the root resorption in this study nevertheless it may have contributed to the forces on the teeth, and thus the amount of resorption, masking the effects of the different force magnitudes used.

9.6.6 Effects of GS and CS on tooth movement and root resorption

The initial results of this study indicate that GS and CS may reduce orthodontically induced root resorption by 50% while not significantly affecting the rate of tooth movement (Figure 16) and (Figure 17). However, it was found that the animals in experimental group had gained less weight than the control animals at sacrifice. This weight differential was found to be statistically significant and may have affected the results. When the weight of the animals was considered as covariate in the analysis it was still found that GS and CS had a tendency to reduce the amount of root resorption but the result becomes only marginally significant. It is difficult to assess whether the weight differential may have contributed to a difference in root resorption especially considering that the difference of the mean weight of the groups was less than 10%. Most studies on similar models have demonstrated weight loss of the animals during the experimental period which may translate to poor welfare of the animals [32, 66, 84]. In this study, animals in both the control and experimental groups gained weight which indicates good animal welfare; therefore it is believed that the weight differential of the animals had no considerable effect on the physiologic tissue response to orthodontic loading. This is also

supported by the fact that the results for the orthodontic tooth movement remained unchanged when weight of the animals was included as a covariate in the analysis. It should also be noted that there were large individual variations in the amount of root resorption between the subjects. Nevertheless it does appear that GS and CS may offer some protection against OIIRR. From the methodology used in this study it is difficult explain the mechanism by which this has taken place but it is likely through an interaction of several mechanisms.

Firstly GS and CS, especially CS, are proteoglycans that are not only present in the articular tissues but they are an integral part of most of the bodies' connective tissues including the PDL, alveolar bone and cementum and play an integral part in the metabolism and remodeling of these tissues [85]. The collagenous fibrous network of the PDL provides structural support and is embedded in and interacting with a non-collagenous matrix that consists of proteoglycans and various glycoproteins [86].

Proteoglycans are comprised of a protein core to which one or more glycosaminoglycan (GAG) chains are attached. Both the protein and the type of GAG are important in determining the function within the extracellular environment. GAG chains are linear and consist of a disaccharide repeating unit of hexouronic acid and an n-acetyl hexosamine (Table 18). According to the repeating disaccharide units there are 7 GAG species 6 of which are sulfated these include: chondroitin 6 sulfate (C6S), chondroitin 4 sulfate (C4S), Dermatan sulfate, heparin sulfate and keratan sulfate [86].

It is believed that the structure of proteoglycans plays an important role in determining their function. This can be seen from the distribution of the various proteoglycans in the periodontium. While dermatan sulfate has been found to predominate in the non-mineralized tissues of the PDL, CS was found to predominate in the alveolar bone and cementum.

Several functions are related to proteoglycans in the PDL. These functions will vary according to the size of the molecules involved [87]. Firstly it is believed that larger molecules including agrecan and versican, which are CS containing macromolecules, regulate the physicochemical properties of the tissues and contribute to the overall structural scaffolding of the extra cellular matrix [87]. The smaller molecules are believed to play a role in collagen fibril formation [86] and regulation of mineralization [88] while they are also reported to play a role in the binding of growth factors [89].

Changes in PDL proteoglycans have been reported with orthodontic tooth movement. Using immunolocalization increased levels of Chondroitin-6-sulfate could be detected near the bone surface corresponding to areas of compression in the non-hyalinized and hyalinized zones of the PDL, whereas that of CH-4S/DS did not appear to be influenced by the mechanical stress [90]. C4S has also been detected on the compression side in the gingival cervicular fluid GCF with experimental canine retraction [91] [92].

GS and CS supplements are used in the management of OA with several mechanisms proposed for their function. It is possible that they may have similar effects on the tissues of the PDL.

Firstly GS and CS have demonstrated anti inflammatory effects by acting on several inflammatory mediators. Inflammatory mediators and cytokines such as prostaglandins, IL-1 and nitric oxide (NO) play an important role in tooth movement and root resorption [1, 5-7] and most studies on NSAIDs have shown them to reduce root resorption albeit with some inhibition of tooth movement [15-17]. Recently two studies on NSAIDS showed some reduction in root resorption without significantly reducing tooth movement [18, 93]. GS and CS reduce the production of IL-1β-induced PGE2 production [50] and the same was for IL-1β-induced NO [54]. It has also been demonstrated that the combination of GS and CS was effective in reducing the gene expression for NO synthase, COX-2 and PGE [52] which may explain their anti inflammatory action. Most studies indicate that the anti-inflammatory properties are stronger when GS and CS are used in combination [40]. It is difficult to explain why the anti inflammatory action is associated with a reduction or inhibition of tooth movement with some anti-inflammatory agents and not with others. It was speculated that selective COX-2 inhibitors may have different effects to those of conventional NSAIDs but studies showed that among subtypes of COX-2 inhibitors the effects on tooth movement were different [16, 17]. It is possible that the anti inflammatory action of GS and CS may play role in reducing orthodontically induced root resorption and may also play a role in reducing pain associated with orthodontic appliance activation.

CS has also been found to reduce the effects of matrix degrading enzymes such as proteases. Matrix metalloproteinases (MMPs) are a particular group of proteinases involved in the remodeling of extracellular matrix components of most connective tissues. MMPs activity is normally in a balance regulated by its specific tissue inhibitors (TIMPs) [55]. In OA patients MMPs activity and the balance with TIMPs is disturbed [94]. CS has been found to decrease the effects of MMPs by down regulating their synthesis as well as reducing their activity thus accounting for its chodroprotective properties [51, 56-58]. It has also been demonstrated that CS reduces the levels of IL-1β-induced extracelluar kinases which may explain some of its anti catabolic effect [50]. MMPs play a vital role in extracellular matrix remodeling in the periodontium incident to tooth movement and root resorption as well [95, 96]. It is believed that modifying the function of these enzymes and their inhibitors can have an effect on tooth movement. A recent study [97] demonstrated that increased TIMP can inhibit tooth movement. It is possible that the GS and CSs' effect on MMPs may also be expressed in the extracellular matrix of the PDL and affect the remodeling of the periodontium incident to orthodontic force application.

Furthermore CS has been shown to have an effect on factors that influence bone metabolism. Three factors have been shown to control bone metabolism namely osteoprotegrin (OPG), receptor activator of nuclear factor kappa (RANK) and its ligand RANKL [98, 99]. The first two are produced by osteoblasts with RANKL being essential for osteoclast differentiation and bone resorption. OPG on the other hand is a decoy

receptor that blocks RANKL therefore preventing it from binding with its receptor RANK on osteoclasts thus inhibiting osteoclast differentiation and bone resorption. The effect of CS on these bone resorption factors has recently been studied on human OA sub-chondral osteoblasts after the stimulation with vitamin D3 [60]. The results showed that CS up-regulated the ratio of OPG:RANKL i.e. it up-regulated OPG and also down-regulated RANKL. Considering that in OA abnormal osteoblasts increase the expression of RANKL and increase bone resorption; CS would have a positive effect on bone protection in OA [40]. Evidence is emerging that RANKL and OPG produced by periodontal ligament fibroblasts and osteoblasts play an important role in regulating tissue turnover and bone resorption during orthodontic tooth movement [100, 101]. One recent study demonstrated that local delivery of OPG can inhibit osteoclast differentiation and bone resorption thus preventing tooth movement [102]. Some evidence suggests that a similar role may take place by cementoblasts in root resorption [101] but evidence is not yet sufficient [103]. It is possible that CS may alter the OPG:RANKL ratio in the PDL thus reducing resorption of cementum.

It is difficult to discern the exact relation ship between GS and CS from the methodology used in this study. The evaluation was limited to volumetric quantification of root resorption and metric evaluation of tooth movement. No histological sections or biochemical analysis were performed. Although the results of this study suggest that GS and CS may offer some protection against orthodontically induced root resorption in rats, further study is required to explain the mechanism of action of these compounds in the PDL before any clinical application can be suggested. If GS and CS are found to play a

role in the prevention or reduction of root resorption they could provide a possible remedy that is readily available as an over the counter nutritional supplement. It would be safe to use and have very little known side effects if used during and after orthodontic treatment. They may also have the potential to reduce pain and discomfort associated with appliance activation.

On the other hand GS and CS are considered to be slow acting drugs SySADOA in the treatment of OA. Their maximal effect is only attained after several months of treatment [69] and they also have a carryover effect that persists after treatment is stopped [40]. Although a period of two weeks was found to be sufficient to produce sufficient effect in rats [62], several months are required in humans to reach a therapeutic effect. This needs to be taken into consideration if any use during orthodontic treatment is contemplated.

9.7 Conclusion

The findings of this study indicate that systemic administration of GS and CS may reduce root resorption incident to orthodontic tooth movement while not affecting the rate of tooth movement, although further research is required to clarify the mechanism of action of these supplements on the periodontium and whether they could potentially be used during the course of orthodontic treatment.

9.8 References

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10 Appendix

10.1 List of Tables

Table 1 Grouping of the animals

	Light continuous force **10 cN (centi Newton)** **On one side**	**Heavy continuous force** **150-160 cN** **On one side**
Experimental groups (GS+CS)	**Group 1** **N=20**	**Group 2** **N=20**
Control groups (regular diet)	**Group 3** **N=20**	**Group 4** **N=20**

Table 2 Weight of animals before and after experiment

Drug	Gp	Weight at start of experiment			Weight at sacrifice		
		Mean	N	Stdev	Mean	N	Stdev
no	no drug, light force	211.217	12	17.8565	247.750	12	13.5247
	no drug, heavy force	203.900	17	16.6444	242.971	17	17.7491
	Total	206.928	29	17.2343	244.948	29	16.0504
GS&CS	drugN, light force	203.583	6	9.5342	217.933	6	13.3425
	drugN, heavy force	207.186	14	14.6919	225.879	14	18.2752
	Total	206.105	20	13.2090	223.495	20	17.0093
Total	drugN, light force	203.583	6	9.5342	217.933	6	13.3425
	drugN, heavy force	207.186	14	14.6919	225.879	14	18.2752
	no drug, light force	211.217	12	17.8565	247.750	12	13.5247
	no drug, heavy force	203.900	17	16.6444	242.971	17	17.7491
	Total	206.592	49	15.5722	236.192	49	19.4497

Table 3 Analysis of weight of the rats before the experiment

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	439.095(a)	3	146.365	.588	.626
Intercept	1793776.317	1	1793776.317	7206.762	.000
Gp	439.095	3	146.365	.588	.626
Error	11200.582	45	248.902		
Total	2102968.840	49			
Corrected Total	11639.677	48			

a R Squared = .038 (Adjusted R Squared = -.026)

Table 4 Analysis of the weight of animals at sacrifice (Dependent Variable: Weight at sacrifice)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5873.595(a)	3	1957.865	7.172	.000
Intercept	2296768.324	1	2296768.324	8413.453	.000
Gp	5873.595	3	1957.865	7.172	.000
Error	12284.442	45	272.988		
Total	2751700.640	49			
Corrected Total	18158.037	48			

a R Squared = .323 (Adjusted R Squared = .278)

Table 5 Univariate Analysis of Variance for the effect of GS and CS and force magnitude on the root resorption volume (Dependent Variable: RRvol)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	327616022.540 (a)	2	163808011.270	4.004	.025
Intercept	2692818757.069	1	2692818757.069	65.817	.000
Drug	326763869.071	1	326763869.071	7.987	.007
Force	1393021.982	1	1393021.982	.034	.854
Error	1882041007.460	46	40913934.945		
Total	5661102031.000	49			
Corrected Total	2209657030.000	48			

a R Squared = .148 (Adjusted R Squared = .111)

Table 6 Estimated Marginal Means (Drug)

Drug	Mean	Std. Error	95% Confidence Interval	
	Lower Bound	Upper Bound	Lower Bound	Upper Bound
no	10505.127	1199.121	8091.422	12918.831
GS&CS	5215.274	1480.327	2235.529	8195.019

Table 7 Estimated Marginal Means (Force)

Force	Mean	Std. Error	95% Confidence Interval	
	Lower Bound	Upper Bound	Lower Bound	Upper Bound
light	7684.136	1539.585	4585.113	10783.159
heavy	8036.265	1152.392	5716.620	10355.910

Table 8 Univariate Analysis of Variance of the effects of GS and CS and Force magnitude on Tooth movement (dependant variable Tooth movement)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1479.788(a)	2	739.894	3.338	.045
Intercept	22497.274	1	22497.274	101.487	.000
Drug	6.499	1	6.499	.029	.865
Force	1464.012	1	1464.012	6.604	.014
Error	9753.770	44	221.677		
Total	40853.566	47			
Corrected Total	11233.558	46			

a R Squared = .132 (Adjusted R Squared = .092)

Table 9 Estimated Marginal Means (Drug)

Drug	Mean	Std. Error	95% Confidence Interval	
	Lower Bound	Upper Bound	Lower Bound	Upper Bound
no GS&CS	23.785 23.016	2.833 3.584	18.076 15.793	29.494 30.240

Table 10 Estimated Marginal Means (Drug)

Force	Mean	Std. Error	95% Confidence Interval	
	Lower Bound	Upper Bound	Lower Bound	Upper Bound
light	17.508	3.728	9.996	25.020
heavy	29.293	2.722	23.806	34.780

Table 11 Univariate Analysis of Variance for the effect of GS and CS and force magnitude on the root resorption volume with weight as covariate (Dependent Variable: root resorption volume)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	370805292.670 (a)	3	123601764.223	3.025	.039
Intercept	7734579.022	1	7734579.022	.189	.666
Drug	133925355.507	1	133925355.507	3.277	.077
Force	1403331.895	1	1403331.895	.034	.854
WtS	43189270.130	1	43189270.130	1.057	.309
Error	1838851737.330	45	40863371.941		
Total	5661102031.000	49			
Corrected Total	2209657030.000	48			

a R Squared = .168 (Adjusted R Squared = .112)

Table 12 Estimated Marginal Means (drug), dependant variable root resorption volume

Drug	Mean	Std. Error	95% Confidence Interval	
	Lower Bound	Upper Bound	Lower Bound	Upper Bound
no GS&CS	9994.582(a)	1297.201	7381.884	12607.280
	5955.141(a)	1645.170	2641.598	9268.684

a Covariates appearing in the model are evaluated at the following values: Weight at sacrifice = 236.192.

Table 13 Estimated Marginal Means (force), dependant variable root resorption volume

Force	Mean	Std. Error	95% Confidence Interval	
	Lower Bound	Upper Bound	Lower Bound	Upper Bound
light	7798.146(a)	1542.624	4691.141	10905.151
heavy	8151.577(a)	1157.129	5821.000	10482.154

a Covariates appearing in the model are evaluated at the following values: Weight at sacrifice = 236.192.

Table 14 Univariate Analysis of Variance for the effect of GS and CS and force magnitude on tooth movement with weight as covariate (Dependent Variable: tooth movement)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1850.234(a)	3	616.745	2.826	.050
Intercept	64.457	1	64.457	.295	.590
Drug	61.760	1	61.760	.283	.597
Force	1498.928	1	1498.928	6.869	.012
WtS	370.446	1	370.446	1.698	.200
Error	9383.323	43	218.217		
Total	40853.566	47			
Corrected Total	11233.558	46			

a R Squared = .165 (Adjusted R Squared = .106)

Table 15 Estimated Marginal Means (Drug) Dependent Variable: Tooth Movement

Drug	Mean	Std. Error	95% Confidence Interval	
	Lower Bound	Upper Bound	Lower Bound	Upper Bound
no	22.332(a)	3.024	16.234	28.430
GS&CS	25.109(a)	3.902	17.240	32.978

a Covariates appearing in the model are evaluated at the following values: Weight at sacrifice = 236.204.

Table 16 Estimated Marginal Means (force) Dependent Variable: Tooth Movement

Force	Mean	Std. Error	95% Confidence Interval	
	Lower Bound	Upper Bound	Lower Bound	Upper Bound
light	17.756(a)	3.703	10.288	25.225
heavy	29.684(a)	2.718	24.203	35.165

a Covariates appearing in the model are evaluated at the following values: Weight at sacrifice = 236.204.

Table 17 Intraoperator error for the measurements of the total volume of root resorption, tooth movement in the sagittal plane and tooth movement in the axial plane

	Root resorption	Tooth movement sagittal	Tooth movement axial
mean	10822.8	35.352222	34.85833
mse	733871.7	0.139711	0.8897166
SE mt	**857**	**0.374**	**0.943**
CV(%)	**7.92**	**1.06**	**2.71**

Table 18 Distribution and function of proteoglycans in the the PDL

Molecular size	Name		distribution and function in the PDL
Large molecular size	Large aggregating proteoglycans	Versican	involved in maintaining tissue hydration/ contribute to the overall structural scaffolding of the extracellular matrix, *Bartold et al 1998*
		Agrecan	mainly soft CT PDL and gingiva
Small molecular size	Small Leucine-rich Proteoglycans (SLRPs)	Biglycan	Biglycan and decorin carrying DS chains predominate in the PDL
		Decorin	
		Fibromodulin	Biglycan and decorin with one or two CS chains predominate in the bone and cementum
		Lumican	
Small molecular size	Cell Surface Proteoglycans	Syndecan	identified on most cell surfaces Cell-cell and cell-matrix interactions, binding of growth factors and cytokines. influences cell adhesion proliferation and differentiation

10.2 List of figures

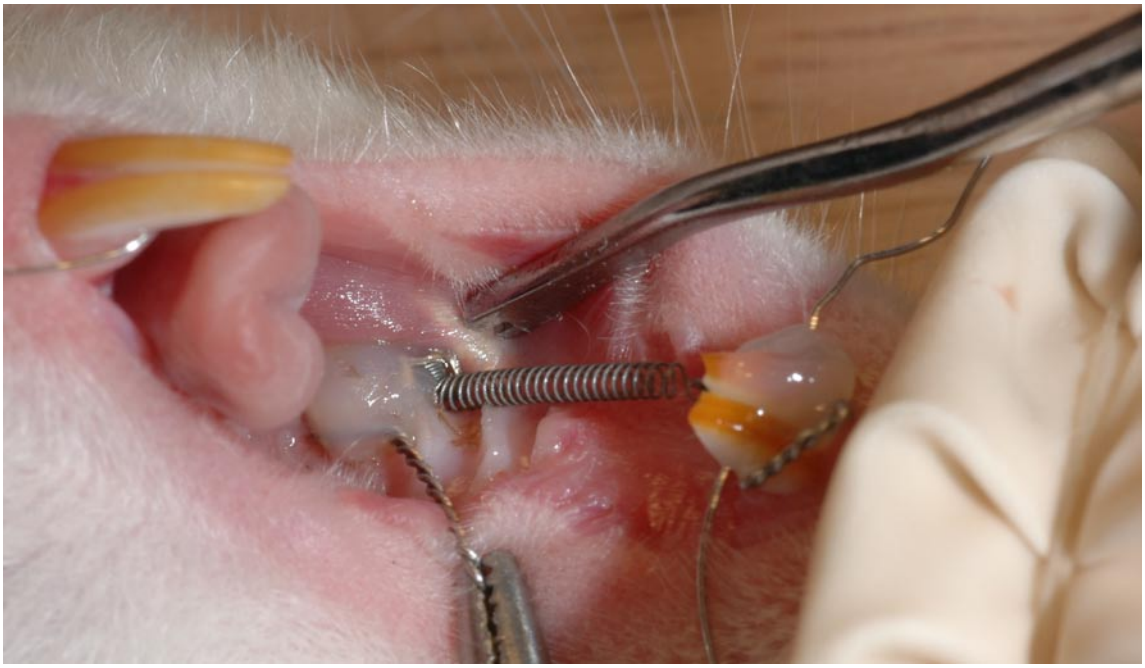

Figure 1 Appliance placement

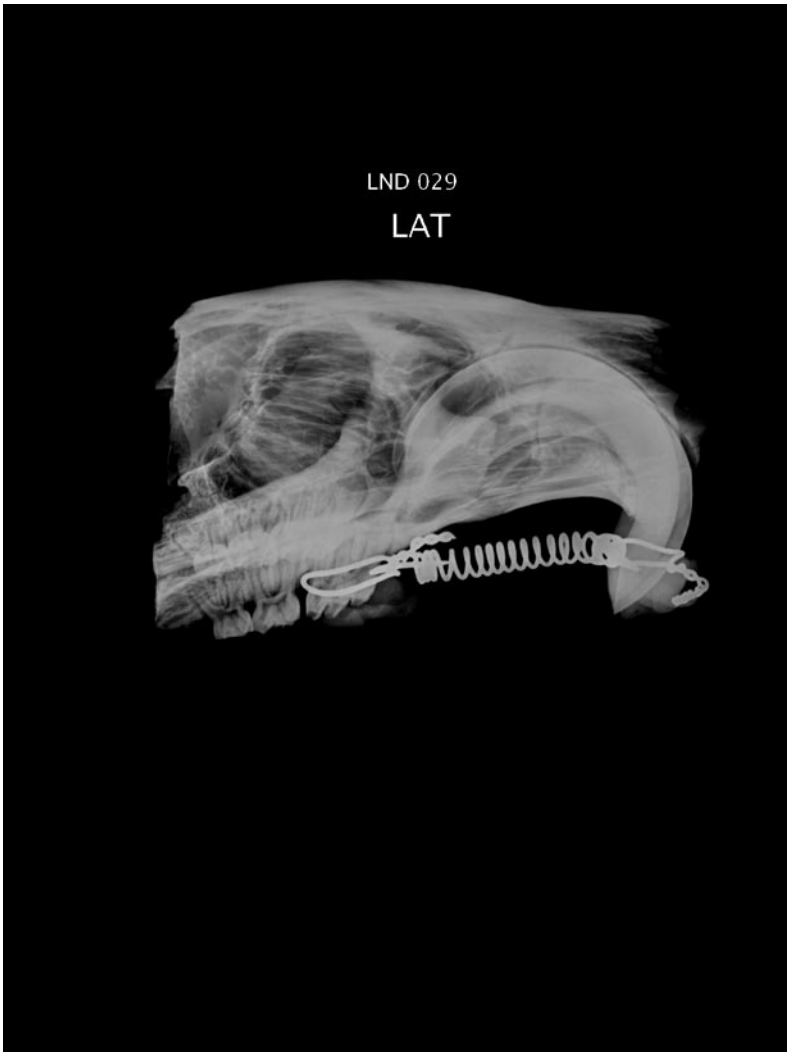

Figure 2 Appliance as viewed from a lateral cephalogram

Figure 3 Skyscan 1172 Desk Top microCT X-ray scanner

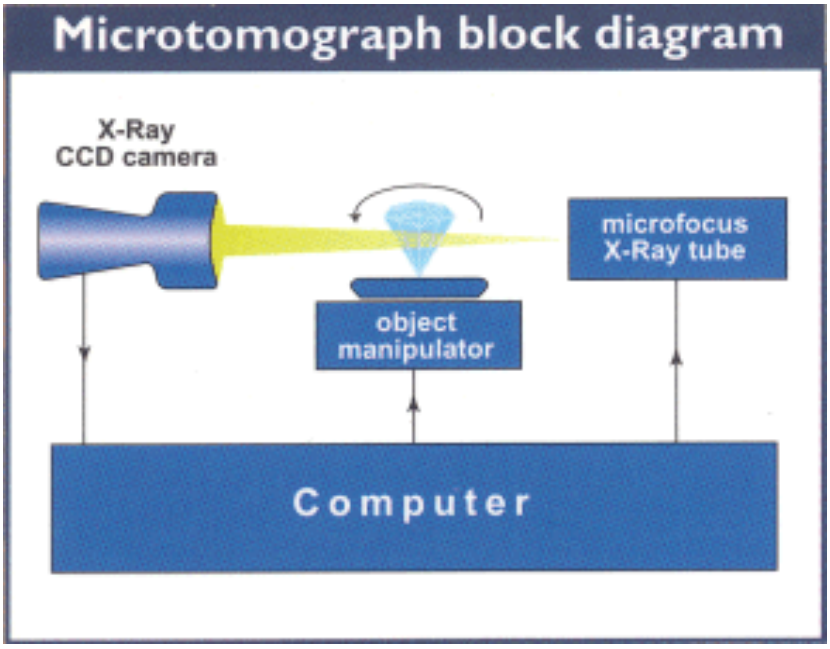

Figure 4 Diagram of the microCT scanner

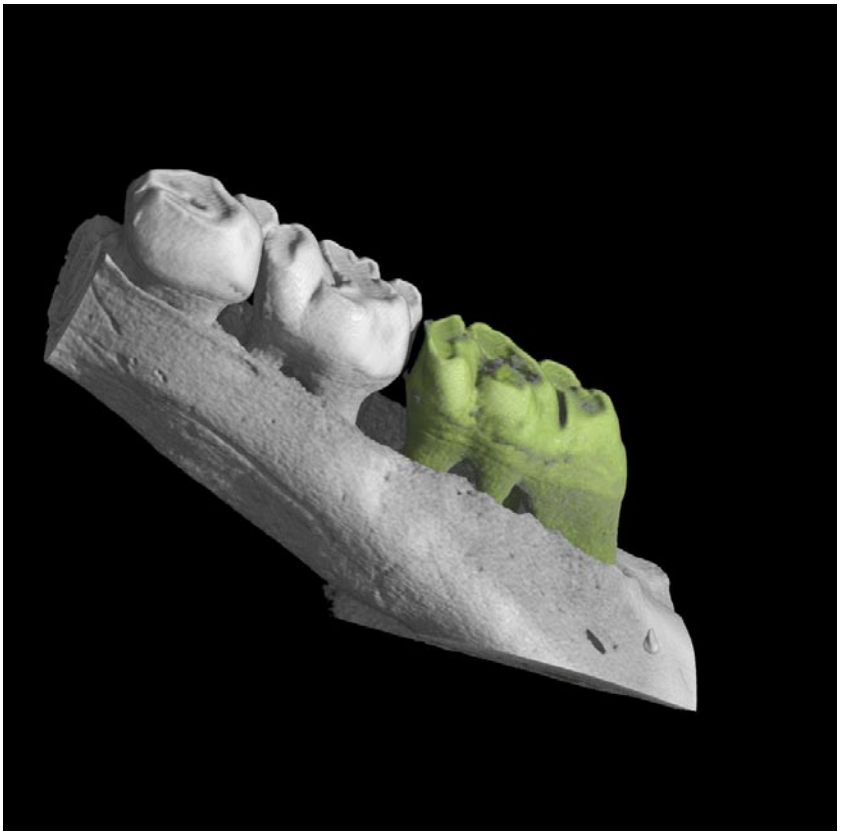

Figure 5 Three dimensional view of rat molar segment with first molar highlighted in green

Figure 6 Screen capture demonstrating digital extraction of the maxillary first molar

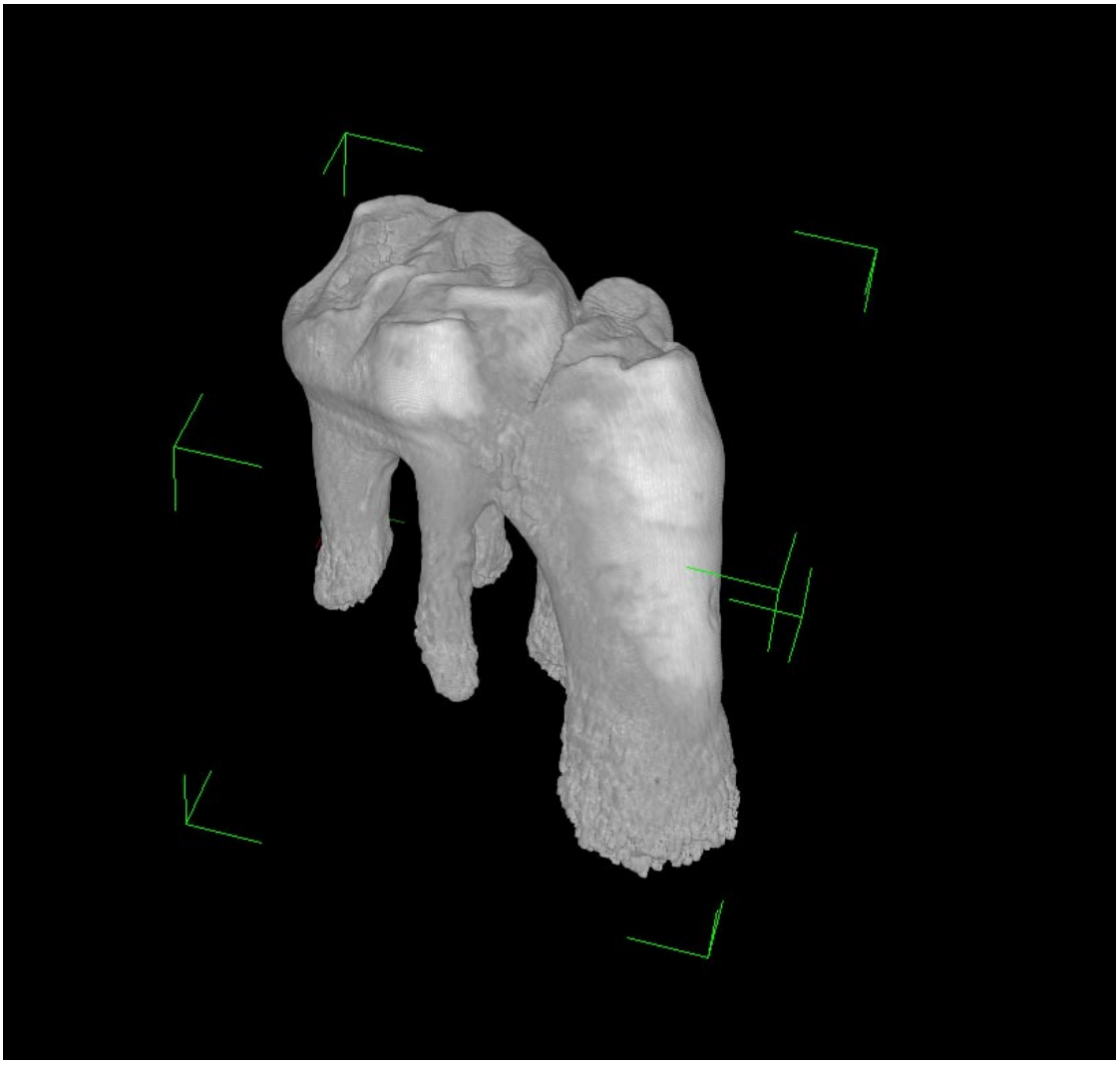

Figure 7 Digitally extracted maxillary first molar on screen view for 3-D analysis

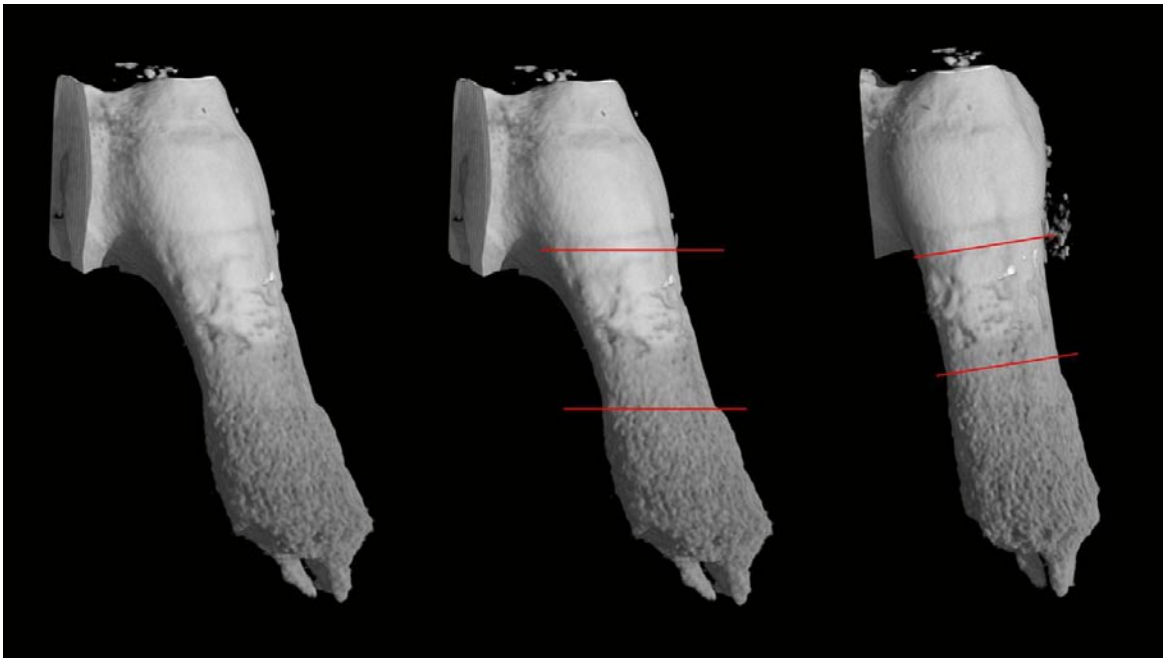

Figure 8 Showing the mesial root of the maxillary first molar. Highlighted between the red lines is the segment of the root analysed for root resorpiton. The porous apical region can be clearly seen in this view.

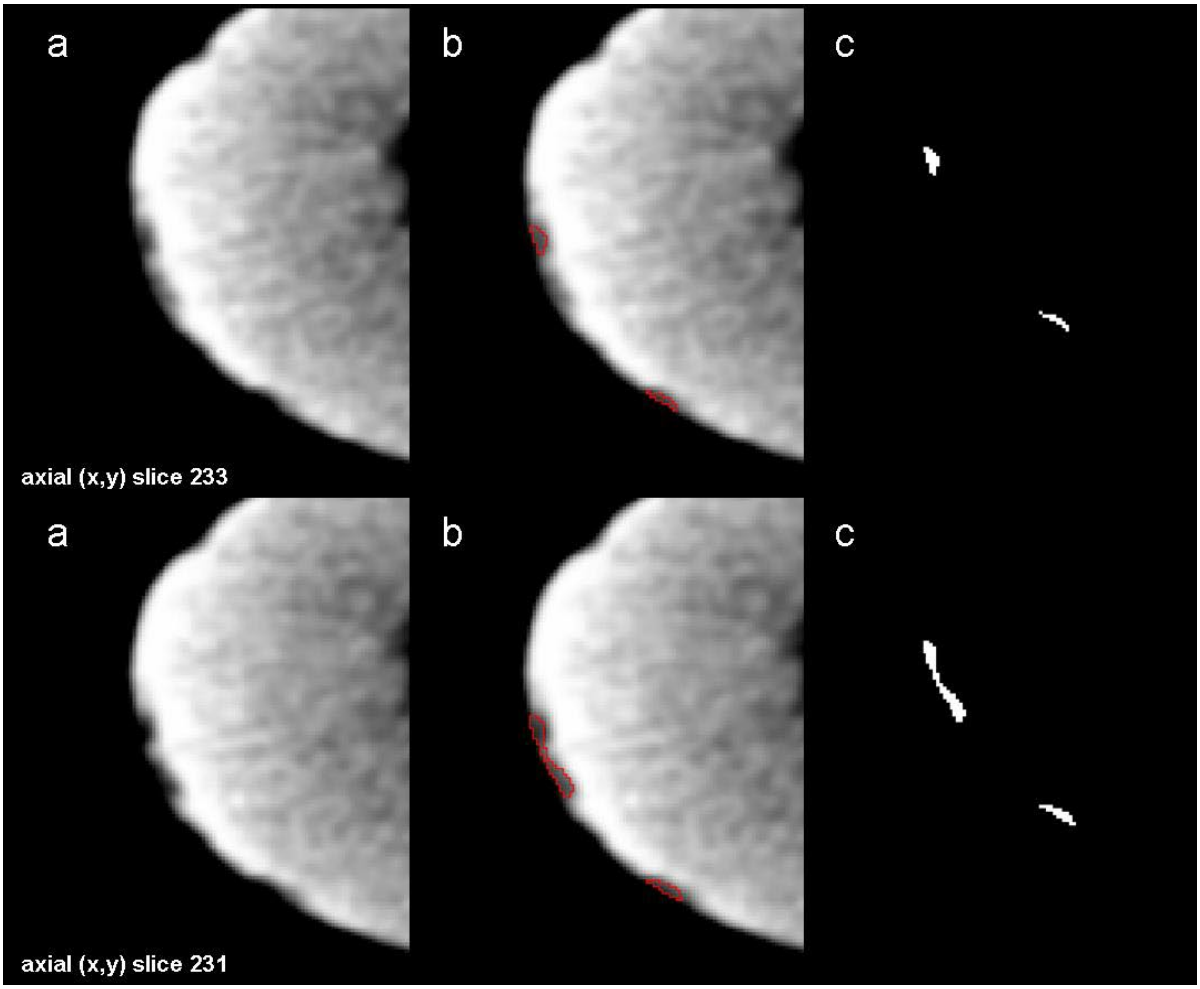

Figure 9 Crater isolation method sections 233-231: Column (a) shows crater in cross section. (b) shows tracing of crater out line. (c) shows isolated crater outline to added for total volume calculation

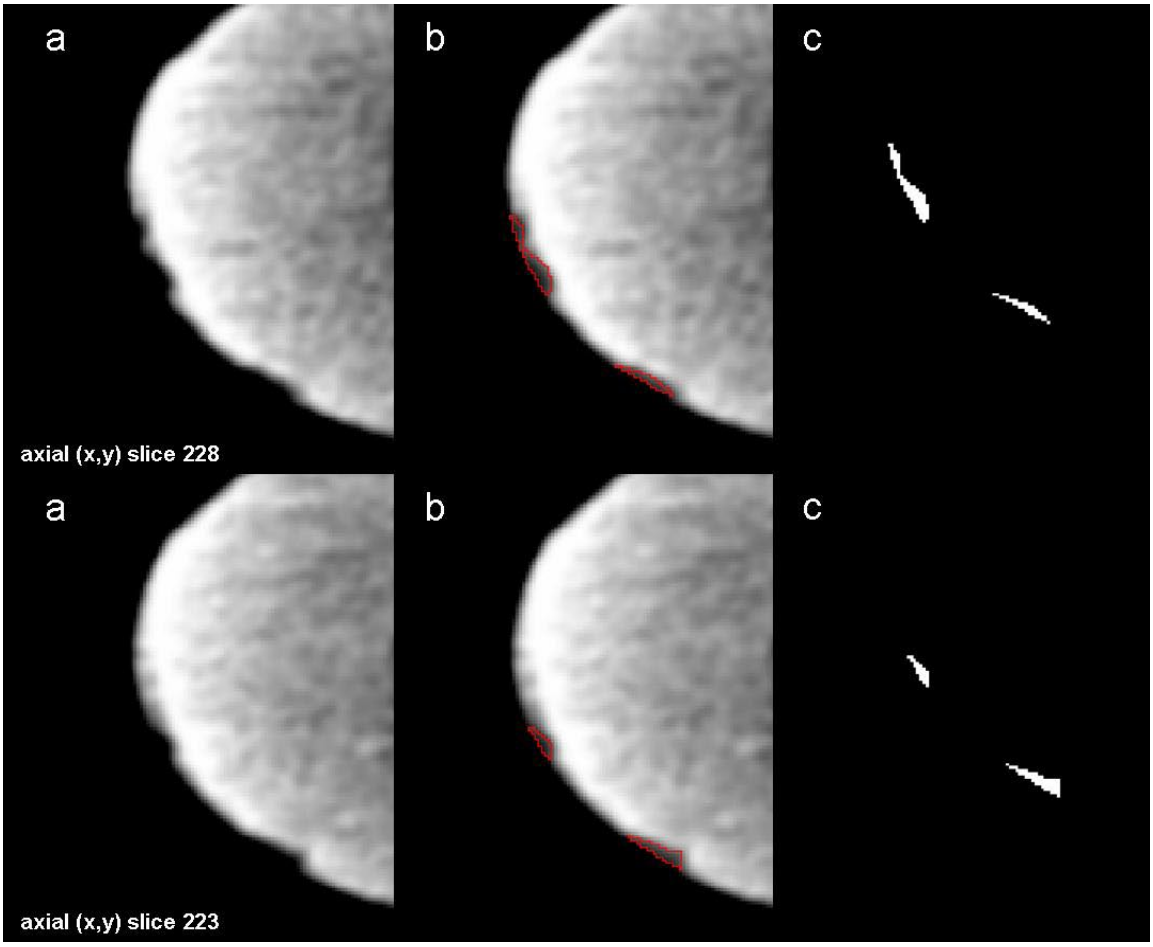

Figure 10 Crater isolation method sections 228-223: Column (a) shows crater in cross section. (b) shows tracing of crater out line. (c) shows isolated crater outline to added for total volume calculation

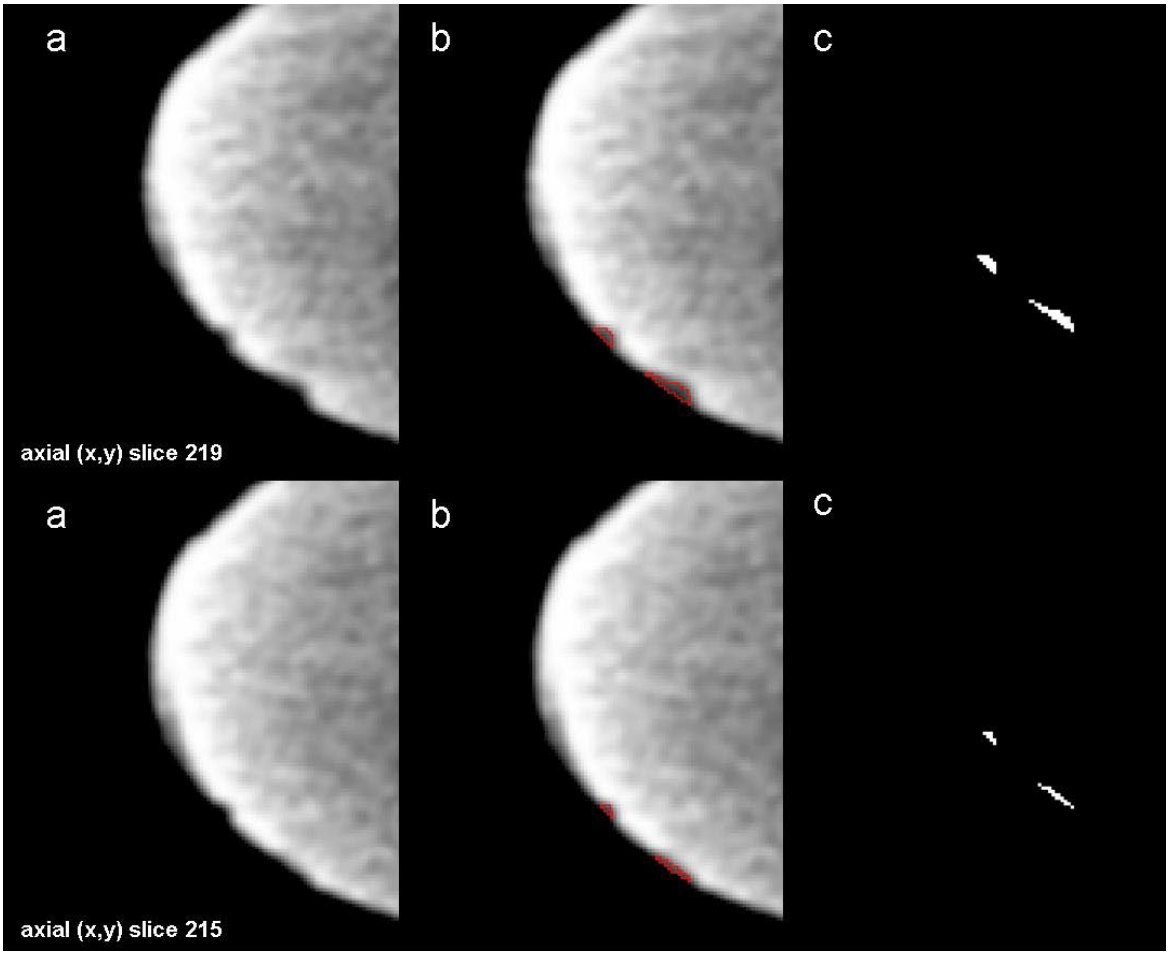

Figure 11 Crater isolation method sections 219-215: Column (a) shows crater in cross section. (b) shows tracing of crater out line. (c) shows isolated crater outline to added for total volume calculation

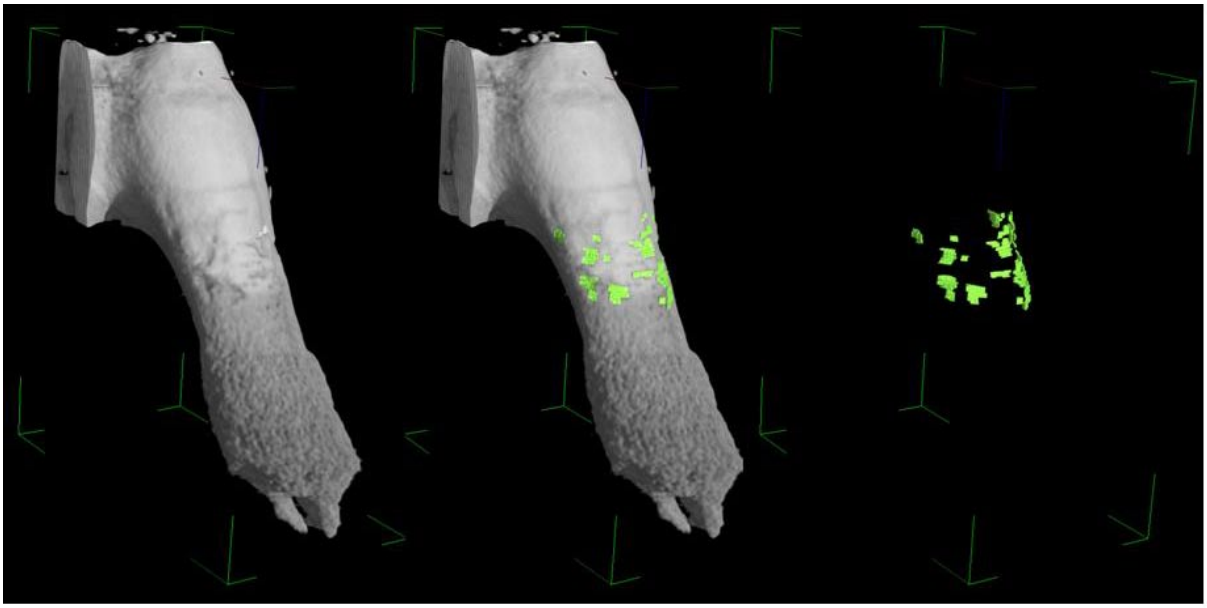

Figure 12 demonstrating the sequence of crater identification and isolation. The volume highlighted in green represents the total volume of root resorption and will be calculated using the software.

Figure 13 Tooth movement measurement. The thin red line between the contacts of the first and second molars represents the shortest distance between the two surfaces as detected by the software tool

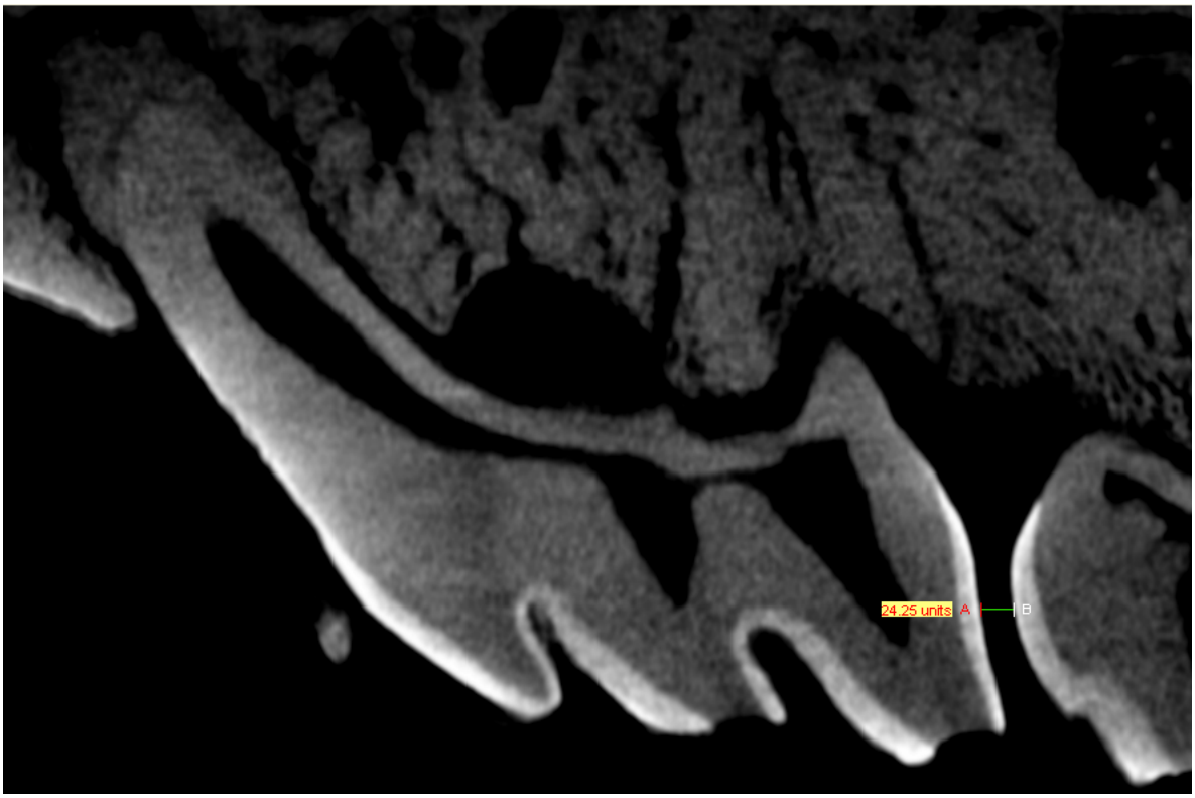

Figure 14 Sagittal section demonstrating the shortest distance between the two surfaces in the sagittal plane

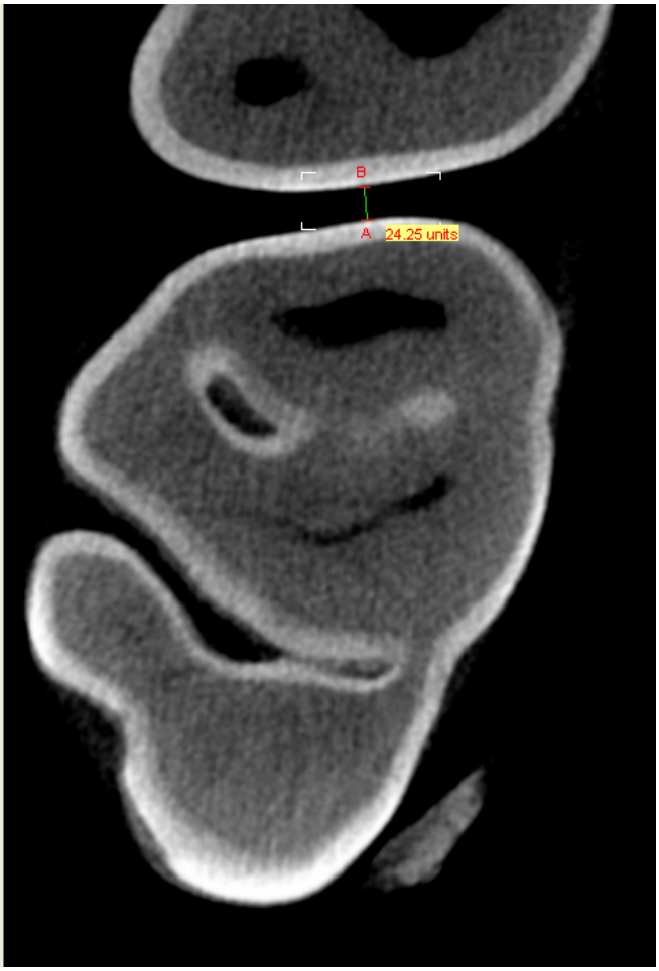

Figure 15 Axial section demonstrating the software tools' measurement of the shortest distance between the two surfaces in the axial plane

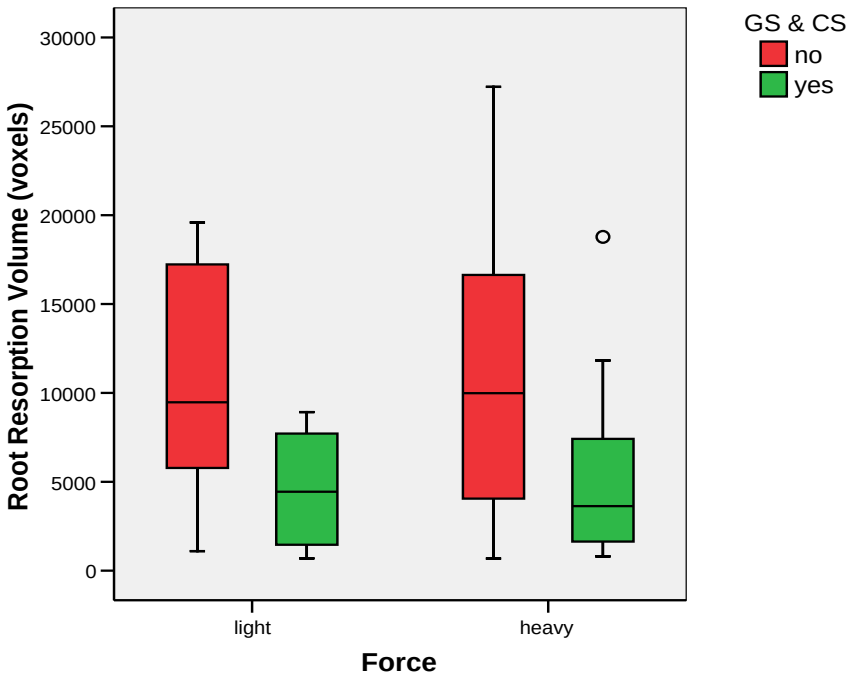

Figure 16 Boxplot graph of root resorption volume in the various groups

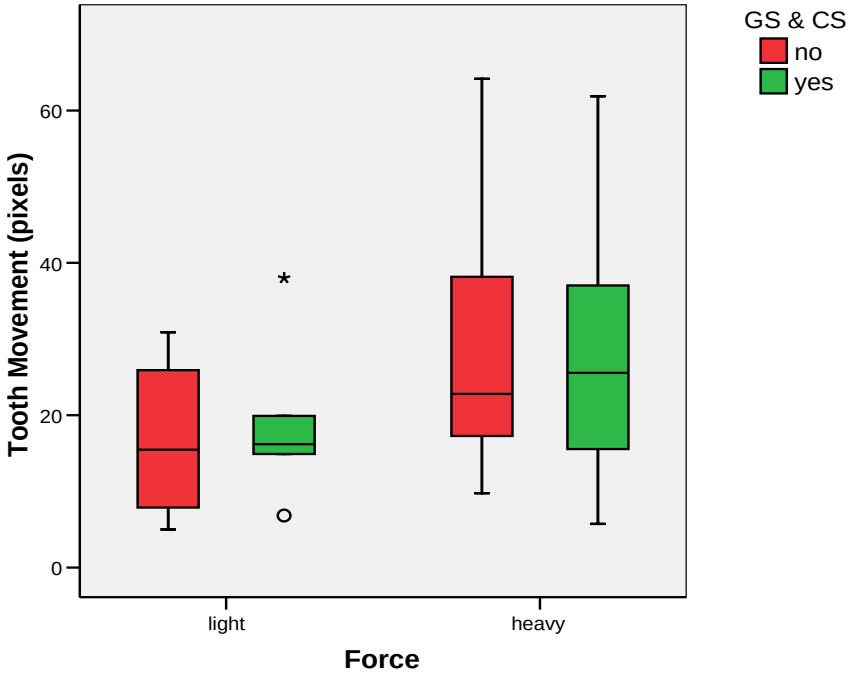

Figure 17 Boxplot graph of tooth movement distance in the various groups

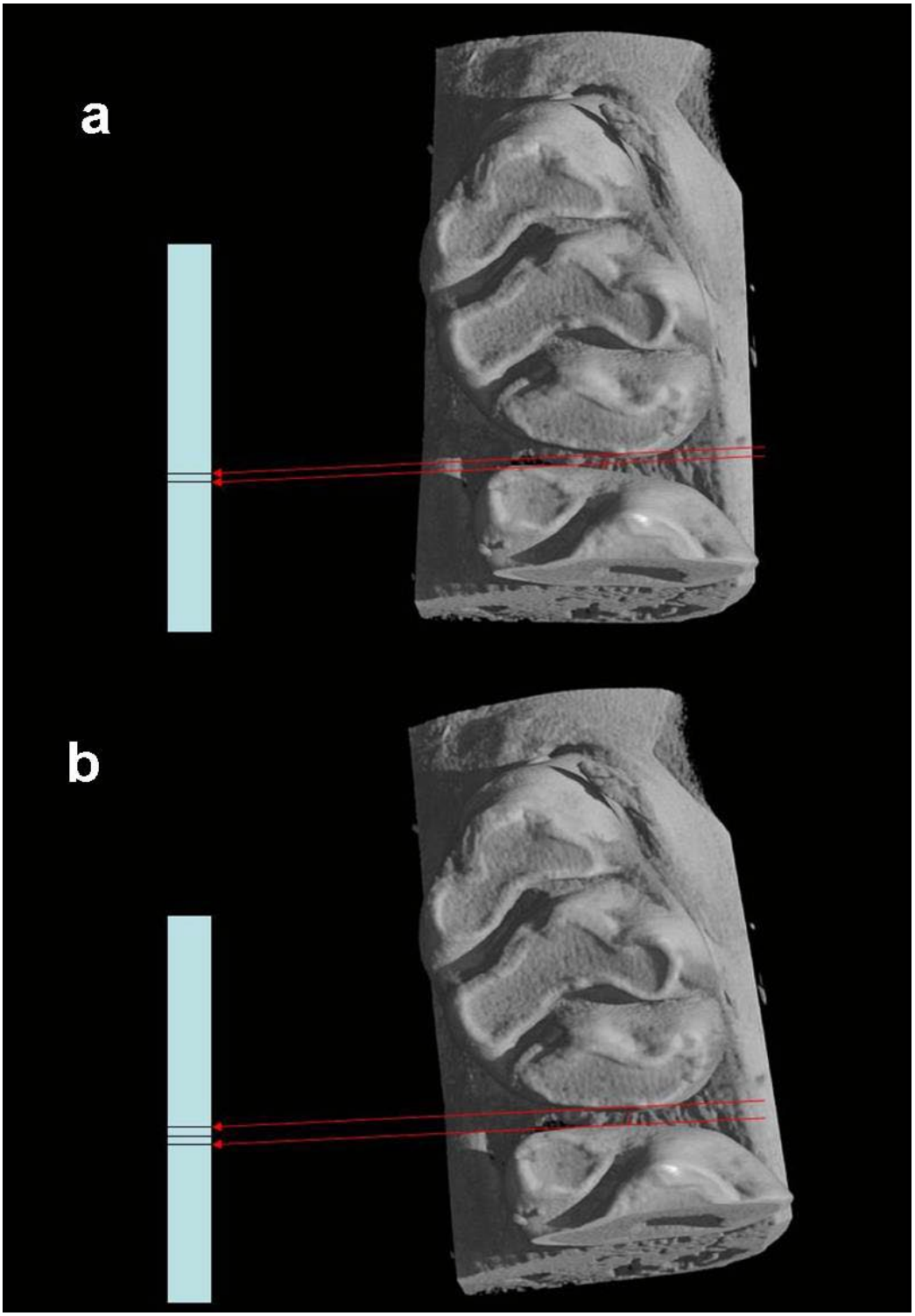

Figure 18 Demonstrating how when the angulations of the beam is changed from (a) to (b) in relation to the contact area the amount of tooth movement measured can be altered

11 Future directions

The results of this study indicated that GS and CS may offer some protection against OIIRR. Further study is required to clarify the mechanisms involved. Only volumetric quantification of the root resorption was performed in this study. Future studies should look into evaluating any changes in the cellular reactions involved in tooth movement and root resorption with GS and CS administration. Histological analysis with immunohistochemistry to evaluate the expression of inflammatory mediators in the PDL after administration of GS and CS may shed light on any similarities to the reactions seen in articular tissues.

Another avenue for future research would be to evaluate any changes in the expression of proteoglycans in the periodontium incident to administration of GS and CS with and without orthodontic loading. It would also shed some light on the exact role of the proteoglycans in the periodontium.

Lastly it is possible that GS and CS may play a role in root resorption repair. It would be interesting to examine whether the connective tissue building properties of GS and CS have any effect on the repair and remodeling of the PDL following cessation of orthodontic force and whether they influence the repair of root resorption lesions.