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### Abbreviations

<table>
<thead>
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<th>Description</th>
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<tr>
<td>1,25 (OH₂) D₃</td>
<td>1,25 dihydroxyvitamin D₃</td>
</tr>
<tr>
<td>2D</td>
<td>Two dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>AEFC</td>
<td>Acellular extrinsic fibre cementum</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>Calcium Carbonate</td>
</tr>
<tr>
<td>CCM</td>
<td>Calcium Citromalate</td>
</tr>
<tr>
<td>CEJ</td>
<td>Cemento Enamel Junction</td>
</tr>
<tr>
<td>CIFC</td>
<td>Cellular intrinsic fibre cementum</td>
</tr>
<tr>
<td>cN</td>
<td>Centinewtons</td>
</tr>
<tr>
<td>CPP</td>
<td>Casein Phosphopeptides</td>
</tr>
<tr>
<td>DC</td>
<td>doxycycline</td>
</tr>
<tr>
<td>EARR</td>
<td>External Apical Root Resorption</td>
</tr>
<tr>
<td>EPMA</td>
<td>Electron Probe Micoranalysis</td>
</tr>
<tr>
<td>ERR</td>
<td>External Root Resorption</td>
</tr>
<tr>
<td>F</td>
<td>Fluoride</td>
</tr>
<tr>
<td>FEN</td>
<td>Finite Element Analysis</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>Micro-CT</td>
<td>Micro-computed tomography</td>
</tr>
<tr>
<td>MMPs</td>
<td>matrix metalloproteinases</td>
</tr>
<tr>
<td>NaF</td>
<td>Sodium Fluoride</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>NiTi</td>
<td>Nickel Titanium</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>OIIRR</td>
<td>Orthodontically induced inflammatory root resorption</td>
</tr>
<tr>
<td>OTM</td>
<td>Orthodontic Tooth Movement</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorous</td>
</tr>
<tr>
<td>Ppm</td>
<td>Parts Per Million</td>
</tr>
<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
</tr>
<tr>
<td>RGD</td>
<td>arginine-glycine-aspartic acid</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>TRAP</td>
<td>Tartrate-Resistant Acid Phosphatase</td>
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</table>
1. Introduction

Root resorption is a common complication associated with orthodontic treatment. Physiological and pathological factors are responsible for a destruction of root structure.

The consequences of root resorption range from slight tooth mobility due to small amounts of root loss to complete tooth loss from excessive amounts of resorption.\(^1\) Radiographically, the resorption may appear as either an apical root blunting, lateral root resorption or in rare cases excessive root loss.

Root resorption may be pathologic or physiologic in nature and it may also occur in association with orthodontic tooth movement. Physiological resorption occurs during the exfoliation of the primary dentition and mesial drifting in the permanent dentition. The mineralized tissues of the permanent dentition are not normally resorbed.\(^2\) Pathological resorption occurs subsequent to a traumatic injury, pathological disease process or iatrogenic causes.

Soon after the advent of radiographic images, evidence demonstrating differences in root morphology before and after orthodontic treatment was presented.\(^3,4\) A study involving a long-term evaluation of upper incisors with severe apical resorption 5 to 15 years following orthodontic treatment showed a significant correlation between tooth mobility, total length and intra-alveolar root length.\(^5\) Apical resorption, however, has been described as less detrimental to the prognosis of a tooth than an equivalent loss of periodontal attachment at the alveolar crest.\(^6\) Kalkwarf et al\(^7\) demonstrated that 4mm of
root resorption translated into 20% of total attachment loss and 3mm apical root loss equals only 1mm crestal bone loss. It is therefore important that periodontal disease is under control in patients with severely resorbed teeth.

Loss of root length also has orthodontic implications due to the coronal movement of the centre of resistance. Less force is required to torque a tooth with a shorter root length. A tooth with a previously shortened root length may therefore be predisposed to further root resorption if forces equivalent to those used with an intact root are applied.

Due to the negative impact that excessive root resorption can have on the prognosis of the dentition, it has been the subject of extensive research. Unfortunately research into the cause of root resorption has been somewhat inconclusive due to the large range of factors that contribute. Furthermore, there has been little success in influencing either the reparative process which occurs following the cessation of force or in the prevention of orthodontically induced inflammatory root resorption.

This review of the literature will seek to outline what is known about the process of root resorption and its association with orthodontic tooth movement. It will describe how root resorption is associated with the loss of mineralized dental tissues and the importance of calcium in maintaining tooth structure. The review will then describe strategies for maintaining calcium balance. Casein Phosphopeptides will then be introduced as a way of promoting calcium availability and their potential to be of benefit in the prevention of orthodontically induced root resorption will be described.
The review will describe how an appropriate way of assessing the potential benefits of casein phosphopeptides is to use an orthodontic tooth movement appliance in a rat model with controlled dietary supplementation.

Finally, the review will describe different methods for analysing root resorption and ultimately how micro computer tomography can be used to gain an accurate, volumetric quantification of root resorption.

Following the review of the literature, an experimental manuscript will be provided to outline the experimental materials and methods, results and conclusions for this project.
2. Tooth Movement

Orthodontic tooth movement occurs following the initiation of an inflammatory process which results in the resorption of bone on the pressure side and deposition of bone on the tension side.

Tooth movement occurs when there is a prolonged application of force that exceeds the bio-elastic limits of tooth supporting structures. The result of this force is an inflammatory reaction in the connective tissues which leads to adaptive remodelling of the periodontal ligament and alveolar bone. Over-compression of the periodontal ligaments leads to a localised ischaemia and cell death, forming an area of necrotic or hyalinised tissue. An attempt is made to remove the hyalinised tissue and initiate repair through a process which resembles sterile inflammation.

Bone remodeling is common to both tooth eruption and orthodontic tooth movement. During masticatory function, the teeth and periodontal structures are subjected to intermittent heavy forces. In orthodontics this force is prolonged in nature and as such it leads to progressive tooth movement.

The inflammatory process which occurs in orthodontic tooth movement is also associated with the destruction and demineralization of the tooth root. Although cementum is more resistant to resorption relative to bone it is still possible for both the cementum and dentine to resorb as a result of this inflammatory process.
3. Cementum

Cementum is a specialized connective tissue that covers the anatomic roots of teeth and shares some physical, chemical and structural characteristics with compact bone.\textsuperscript{23} It is the surface for attachment of collagen fibres that bind the tooth to surrounding structures.

Cementum is different from bone in that it is not innervated, exhibits little or no remodelling and is avascular. In fully matured teeth, cementum contains about 45% to 50% inorganic material and 50% to 55% organic material and water.\textsuperscript{23} The inorganic material consists of calcium and phosphate in the form of hydroxyapatite. Numerous trace elements are found in cementum with fluoride being by far the element with the highest concentration.\textsuperscript{24,25} Protein extracts of mature cementum promote cell attachment, migration and stimulate synthesis of gingival fibroblasts and periodontal ligament cells.\textsuperscript{26}

Cementum is thinnest at the cementoenamel junction (20 to 50 µm) and thickest towards the apex (150 to 200 µm),\textsuperscript{23} although it may exceed 600µm.\textsuperscript{27} Cementum continues to increase in thickness with age.\textsuperscript{28-30}

Two kinds of cementum can be seen with light microscopy. These are acellular and cellular cementum.\textsuperscript{23,27} Acellular cementum covers the root dentine from the cementoenamel junction to the apex, but it is often missing in the apical third of the root. Cellular cementum is located mainly at root apices and contains cementocytes in its lacunae.
Ten Cate\textsuperscript{31} classified cementum according to the time of formation (primary or secondary), the presence or absence of cells within its matrix (acellular or cellular) and the origin of collagenous fibres of the matrix (intrinsic or extrinsic)

a. Acellular Afibrillar Cementum
b. Primary Acellular Extrinsic Fibre Cementum
c. Primary Acellular Intrinsic Fibre Cementum
d. Secondary Cellular Intrinsic Fibre Cementum
e. Secondary Cellular Mixed Fibre Cementum
f. Cellular Mixed Stratified Cementum
g. Intermediate Cementum

\subsection*{3.1 Dentine}

Dentine is approximately 70\% inorganic, 20\% organic and 10\% water.\textsuperscript{32} The organic component is predominantly type I collagen while the inorganic component is predominantly hydroxyapatite. Dentine is yellowish in colour because of the random arrangement of the hydroxyapatite crystals.

Dentin and pulp are formed from the dental papilla and are developed from ectomesenchymal cells.\textsuperscript{32} The formation of dentine starts with odontoblasts secreting a matrix which is high in type I collagen.\textsuperscript{32} The collagen network acts as a supporting scaffold for the formation of hydroxyapatite crystals.
The odontoblasts modulate the local environment within the pre-dentine to produce conditions conducive to mineralisation.\textsuperscript{32} The presence of odontoblastic processes is vital to the slow but continuous requirement for dentine to be turned over.\textsuperscript{32}

The matrix located immediately adjacent to the odontoblasts is not mineralised and it is usually 15-20 µm thick.\textsuperscript{32} This layer is known as pre-dentine. Dentine grows in thickness on average 4µm per day.\textsuperscript{32} It is important to note that dentine is highly porous and medicaments or antigens that gain access to the dentine have direct access to the pulp.\textsuperscript{32} Dentine may be involved in root resorption if the process penetrates the cementum.\textsuperscript{33}
4. Root Resorption

4.1 Definition and Classification

Root resorption is a physiologic or pathologic process that results in a loss of substance from dentine or cementum.\textsuperscript{34} Physiological root resorption occurs during the exfoliation of deciduous teeth. Pathological root resorption of permanent teeth may be either internal or external in origin. Internal root resorption is initiated from within the pulp while external root resorption arises from the periodontium affecting the external surface of the tooth.

External root resorption has been classified by Tronstad\textsuperscript{35} into transient inflammatory resorption and progressive inflammatory resorption. In transient inflammatory root resorption root damage is minimal and the stimulation for the resorptive process is of a short duration while in progressive inflammatory resorption, changes in root structure can be observed radiographically.\textsuperscript{35} Brezniak and Wasserstein expanded the classification by including orthodontically induced inflammatory root resorption (OIRR) for external root resorption (ERR) that is caused by orthodontic force.\textsuperscript{36}

OIRR occurs as a result to the inflammatory process involved in orthodontic tooth movement. It occurs on the cemental surface of the tooth root. Although cementum is more resistant to resorption relative to bone it is still possible for both the cementum and dentine to resorb as a result of this inflammatory process.
4.2 Prevalence

Root resorption has been reported in populations that are orthodontically treated and untreated. Most resorption related to orthodontic tooth movement is clinically insignificant and causes minimal changes to the root length.\textsuperscript{37,38} There is however a high risk group where severe resorption may occur. This group comprises one to three percent of the population.\textsuperscript{39-41}

4.3 Aetiology

Orthodontic root resorption appears to be multifactorial in aetiology. The factors implicated can be divided into environmental factors and mechanical factors. Environmental factors can be further divided into systemic factors and local factors.

There is a high degree of individual susceptibility associated with OIIRR. The incidence varies amongst persons and within the same person at different times.\textsuperscript{34} It also occurs in varying degrees in different teeth.\textsuperscript{42,43}

There are a number of factors that have been implicated as risk factors in OIIRR. There is some evidence for an increase in root reo rption being associated with such things as asthma and allergy,\textsuperscript{43-46} excessive alcohol consumption\textsuperscript{45} and deficiency in dietary calcium and vitamin D.\textsuperscript{47}
4.3.1 Systemic Factors

4.3.1.1 Genetic Factors

Harris et al found a hereditary component to external apical root resorption following a study amongst siblings which suggests that genetic factors play a role in EARR.\textsuperscript{48} Al Qawasmi et al identified a key role of the IL-1\(\beta\) gene polymorphism for a genetic influence in EARR in orthodontically treated individuals.\textsuperscript{49} They showed a 5.6 fold risk of external apical root resorption of greater than 2mm in polymorphisms which were homozygous for the IL-1\(\beta\) allele 1.\textsuperscript{49,50} This polymorphism accounted for approximately 15\% of variation in EARR of upper centrals.\textsuperscript{48} Harris et al\textsuperscript{48} and Hartsfiled et al\textsuperscript{51} deduced that since half of the variation in EARR was influenced by genetic factors, and the variation at IL-1\(\beta\) accounts for only 15 per cent of phenotypic variation, there must be other genes that influenced EARR.

4.3.1.2 Ethnicity

Sameshima and Sinclair\textsuperscript{52} studied a sample of 868 patients and found that Asian patients experienced less root resorption compared to white or Hispanic patients. However this sample population was disproportionate when comparing the Asian and African American samples to the larger Caucasian sample and therefore these results are inconclusive.\textsuperscript{53}
4.3.1.3 Chronological Age

Reduced vascularity, plasticity and width of the periodontal ligament, increased density and reduced vascularity of bone and an increase in the width of cementum are associated with increasing age. Reitan suggested that these changes are responsible for the increased incidence of root resorption seen in adults.\textsuperscript{54}

4.3.1.4 Dental Age

Partially formed roots appear to develop normally during orthodontic treatment and it has been suggested that teeth with open apices may be more resistant to apical root resorption\textsuperscript{41,55,56}.

4.3.1.5 Endocrine Imbalance

Altered endocrine conditions such as hyperparathyroidism,\textsuperscript{47,57} Paget’s disease,\textsuperscript{58} hypophosphataemia,\textsuperscript{59} hypothyroidism, hypopituitarism and hyperpituitarism\textsuperscript{60,61} are associated with altered resistance to root resorption. This can be explained by the control of the metabolism of mineralised tissues by the endocrine hormones 1,25-Dihydroxycholecalciferol, parathyroid hormone and calcitonin.\textsuperscript{62}
4.3.1.6 Nutrition

Engstrom demonstrated root resorption in animals deprived of dietary calcium and vitamin D. However, Linge and Goldie and King suggested that nutritional imbalance is not a major factor in root resorption during orthodontic treatment.

4.3.1.7 Alveolar bone density and Turnover

There is some indirect evidence for a potential association between bone density and root resorption. Orthodontic movement of teeth in close proximity to dense cortical bone has been shown to cause greater levels of root resorption than the movement of teeth in trabecular bone. Studies using calcium-deficient rats exhibiting very low alveolar density have demonstrated markedly low levels of root resorption following tooth movement.

4.3.2 Local Factors

4.3.2.1 Habits

The large, non physiological forces produced by habits are similar in nature to the forces produced by orthodontic treatment. An increased risk of external apical root resorption has been noted to be associated with nail biting, finger sucking beyond 7 years of age, and lip or tongue dysfunction.
4.3.2.2 History of trauma

Andreasen has suggested that the initiation of orthodontic force on a traumatised tooth may aggravate the resorptive process. A study by Linge and Linge supported this suggestion through their findings which showed that traumatised teeth lost an average of 1.07 millimetres of root structure compared to 0.64 millimetres in non traumatised teeth.

4.3.2.3 Occlusal Trauma

Occlusal trauma associated with improper occlusion, dental restoration and poorly designed prosthetic appliances have been associated with accelerated root resorption.

4.3.2.4 Root Resorption Prior to Orthodontic Treatment

There is a greater risk of developing further OIIRR in patients with pre-existing evidence of root resorption.

4.3.2.5 Endodontically treated teeth

A tooth with healthy surround tissues which has been treated successfully with endodontics is no more susceptible to resorption than a normal tooth. In fact, Mirabella and Artun and Thilander et al found endodontically treated teeth had less resorption.
Remington et al\textsuperscript{75} have suggested that an increased density of dentine in root filled teeth provides resistance to resorption. On the other hand, a tooth which is in the presence of an active inflammatory process may be more susceptible to root resorption as orthodontic tooth movement itself creates an inflammatory response that may increase the resorptive process.

### 4.3.2.6 Malocclusion

Increased overjet and openbite malocclusions have been implicated as a risk factor for root resorption.\textsuperscript{64,73,76,77}

### 4.3.2.7 Root Morphology

Abnormal or dilacerated roots have been implicated as factors which influence the severity of root resorption. In particular, thin pipette shaped roots have been suggested as being particularly susceptible to root resorption.\textsuperscript{38,78}

### 4.3.2.8 Hypofunctional Periodontium

A hypofunctional periodontium refers to the narrowing of the periodontal space and derangement of functional fibres. This eliminates the normal cushioning effect of the periodontal ligament and results in a high concentration of force which leads to
stimulation of inflammatory mediators which induce the destruction of tooth and bone.\textsuperscript{79,80}

Studies by Sringkarnboriboon et al and Terespolsky et al have demonstrated greater levels of resorption in teeth with hypofunctional periodontium and a delay in recovery of the PDL following orthodontic force. These results suggest that orthodontic movement of non-occluding teeth should be performed with caution.\textsuperscript{81,82}

\subsection*{4.3.2.9 Specific Tooth Vulnerability to Root Resorption}

The teeth most frequently affected by OIIRR in order of severity are the maxillary lateral incisors, maxillary central incisors, mandibular incisors, distal root of mandibular first molar, mandibular second premolars and maxillary second premolars.\textsuperscript{77,83-85} It has been suggested that maxillary lateral incisors are more prone to root resorption due to their abnormal root shape while maxillary incisors in general are more susceptible to root resorption because they are frequently moved greater distances than other teeth.\textsuperscript{77}

\subsection*{4.3.2.10 Abnormal root morphology}

Teeth with short apices, pipette-shaped roots, pointed roots, dilacerated roots and slender roots have all been associated with increased levels of root resorption.\textsuperscript{38,77,86-88} Blunt roots were implicated by root resorption by Levander and Malmgren\textsuperscript{86} and
Thongudomporn and Freer. However this is contradicted by the findings of Sameshima and Sinclair who found that teeth with blunted roots had the least resorption.

Oyama et al explained the increased levels of root resorption in short, bent and pipette roots in an FEN study which demonstrated greater loading of forces in the root apices of these teeth compared with normal teeth.

Teeth with increased root lengths have also been implicated as being at risk of increased root resorption. This is due to the requirement for the use of larger forces to move the teeth and the larger displacement of the root apices during tipping and torquing.

**4.3.3 Treatment related factors**

**4.3.3.1 Duration**

Most studies agree that the risk and severity of external apical root resorption increases as the duration of orthodontic treatment increases. Sameshima and Sinclair looked at a sample of 868 patients collected from 6 different specialist practitioners and found longer treatment times to be significantly associated with increased root resorption for maxillary central incisors. The reasons for the longer duration in treatment may also have had an influence on the increased levels of root resorption seen in these patients.
4.3.3.2 Appliance type

Fixed appliances have been shown to cause more root resorption than removable appliances which can be explained by the increased range of tooth movement afforded by fixed appliances. The risk of root resorption associated with different bracket designs has yielded inconclusive results.

4.3.3.3 Treatment Mechanics

Linge and Linge suggested that the use of inter maxillary elastics increased the amount of root resorption but Sameshima and Sinclair did not find any correlation. No difference has been found between the use of sectional and continuous mechanics. It is generally agreed that the use of rapid maxillary expanders is associated with increased levels of root resorption.

4.3.3.4 Magnitude of applied force

Both human and animal studies agree that there is an increase in severity of root resorption with increasing force magnitude.

Harry and Sims used a scanning electron microscope to examine extracted human premolar teeth that had 50g, 100g and 200g of intrusive force. They concluded that higher forces increased root resorption through an increase in the stress to the root surface which increased the rate of lacunae development.
More recent studies have confirmed the findings of previous studies showing that higher forces increased the amount of external root resorption. Chan and Harris used a volumetric analysis of resorption craters on extracted human teeth to compare controls with a force of 25g or 225g, with buccal displacement\textsuperscript{121} or intrusion.\textsuperscript{122}

Reitan\textsuperscript{123}, on the other hand, found that external root resorption was poorly correlated to force magnitude. He examined 72 premolars after application of 25g to 240g of intrusive, extrusive and tipping movement over a period of 10 to 47 days. A series of studies by Owman Moll et al\textsuperscript{124,125} agreed with the findings of Reitan. They looked at tooth movement with regard to force magnitudes of 50g, 100g and 200g. They found that there was a large inter individual variance but no significant differences in the frequency and severity of root resorption could be detected. They concluded that root resorption was independent of force magnitude, but that individual reactions may be more important.

4.3.3.5 Direction of tooth movement

Intrusion has been consistently implicated as the most likely type of tooth movement to cause root resorption.\textsuperscript{123,126,127} Reitan\textsuperscript{128,129} and Thilander and colleagues\textsuperscript{74} suggested that the stress distribution associated with tipping movements is more likely to cause root resorption than the stress distribution associated with bodily movement.
4.3.3.6 Duration of force application

Debate exists as to whether more root resorption is associated with continuous or intermittent forces. Many believe that discontinuous forces produce less root resorption because the pause in tooth movement allows the resorbed cementum to heal. 129-136

Acar et al135 examined 22 human teeth. The patients were exposed to a continuous tipping force of 100g on one side and an intermittent force applied through elastics for 12 hours per day on the other side, over a period of 9 weeks. Their results showed that the intermittent forces resulted in less root resorption. The accuracy of these results is questionable because the intermittent forces were subject to patient compliance.

Weiland136 studied 84 premolars from patients which had been moved buccally with an orthodontic appliance. On one side of the mouth, force on the premolar was applied with a stainless steel wire (0.016 inch) while force on the contralateral premolar was applied with a super elastic wire (0.016 inch). Their results support the findings of Acar et al137 that continuous forces cause more resorption. They showed that the teeth activated with the super elastic wire moved significantly more, but had 140% more resorption than the teeth with stainless steel wire.

Contrary to these reports Owman Moll et al125 found no difference in the amount or severity of root resorption between forces applied continuously or intermittently after application of a buccally directed force of 50g to human premolars.
4.3.3.7 Amount of tooth movement

Sameshima and Sinclair\textsuperscript{53} found that severe root resorption occurred in their samples when the root apex was displaced lingually, a mean difference of 1mm more than the control group. They concluded that root resorption is directly related to the distance moved by the tooth roots.\textsuperscript{77} Maxillary incisors tend to be moved more than other teeth in orthodontic treatment and therefore this is a possible explanation for why maxillary incisors are at a high risk of root resorption.

4.3.3.8 Extraction protocols

Treatment plans involving extractions were not found to be associated with an increase in the severity of maxillary incisor root resorption. An explanation for this is that the movement of maxillary incisors is not excessive if teeth have been extracted for severe crowding unlike in cases where maxillary incisors are moved large amounts to facilitate the correction of overjet.

4.4 Orthodontic Relapse and Orthodontically Induced Inflammatory Root Resorption

Following appliance removal, there is a conversion of the former pressure side of the active treatment period into the tension side during the relapse period.\textsuperscript{138} This was demonstrated in a study by Langford and Sims\textsuperscript{139} which demonstrated that relapse forces
were capable of causing significant root resorption for up to three months after RME. Consequently it has been recommended that retention with fixed attachments be applied with caution as occlusal trauma to the fixed teeth may lead to continued resorption.\textsuperscript{140}

4.5 Mechanism of Root Resorption

Brudvik and Rygh\textsuperscript{141} studied the histological changes seen under light microscopy of rat and mice molar teeth that had undergone orthodontic tooth movement. They found that the first resorptive attack on the root surface occurred in the area of the over compressed zone. Root resorption occurred in the circumference of the hyalinized tissue after 2 to 3 days in their rat samples. Mononucleated cells derived from adjacent healthy periodontal membrane are implicated in the initiation of resorption of the unmineralized cementoid. Brudvik and Rygh\textsuperscript{142} studied the penetration of cells into precementum and mineralized cementum using transmission electron microscopy. Mononucleated non-clast cells were found during the initial removal of precementum and mineralised acellular cementum at the periphery of the hyalinized tissue. They were also found to be removing precementum and cementum at some distance from the hyalinised tissue. Macrophage-like cells phagocytosed necrotic tissue in the periodontal ligament after 6 hours and near the root surface after 24 hours. Fibroblast-like and cementoblast-like cells are involved in the resorptive process of precementum after 24 hours. The surface layers of mineralized cementum are removed by mononucleated cells after 3 days. Multinucleated cells were seen in surrounding tissues but not usually near the mineralized cementum surface during their 5 day test period.
Brudvik and Rygh\textsuperscript{143,144} investigated further into the resorptive processes beneath the main hyalinized zone. They found that the majority of cells involved in the resorptive process beneath the hyalinised zone were multinucleated. They found that the cells involved in the removal of the main part of the necrotic periodontal membrane, as well as the root surface tissue, are different from that involved during the initial phase. Mononucleated giant cells with ruffled borders were never observed near the remnants of necrotic tissue, but were found in the resorption lacunae of root and bone surfaces. Cementum fragments were not found inside these cells and they postulated that removal of cementum tissue occurred extra cellularly. They also suggested that the multinucleated cells are derived from monocytes and macrophages that initially invaded the necrotic hyalinized tissues.

**4.6 Repair**

Studies in both human and animal subjects have demonstrated repair of root resorption craters following a cessation in orthodontic tooth movement. The risk of tooth loss following orthodontic therapy is not high because a reparative process in the periodontium commences when the applied orthodontic force is discontinued or reduced below a certain level.\textsuperscript{34,54,70,145,146}
4.6.1 Phases of repair

Repair has been recorded as early as the first week of retention.\textsuperscript{147} Harry and Sims\textsuperscript{98} on the other hand documented a longer delay in repair where the appearance of cellular repair cementum in the apical regions occurred at 70 days. Barber and Sims\textsuperscript{148} studied external root resorption in humans following rapid maxillary expansion. They found that subsequent to expansion, repair was the predominant process. However continuing resorption was apparent even after 9 months of retention.

Vardimon \textit{et al}\textsuperscript{138} described the phases of root resorption repair. The incipient phase (14 days) was a transitional stage from no apposition (lag phase) to active deposit stages of repair cementum. The peak phase occurs between 14 to 28 days and is characterized by a spurt in matrix formation. In this phase an initial incorporation of extrinsic fibres into the intrinsic cementum matrix suggests a development towards functional repair. This is followed by a steady deposit of mixed fibrillar cementum between 42 to 56 days.

4.6.2 Clinical implications of repair

A long term radiographic evaluation of root resorption by Remington \textit{et al}\textsuperscript{75} demonstrated progressive remodeling of the root surface after active orthodontic tooth movement. The original root contours and lengths were never re-established despite progressive rounding of jagged, resorbed edges. In their study of 100 subjects only two patients had hypermobility which was considered to be the worst outcome. A review by
Vlaskalic et al\textsuperscript{106} found no reports of tooth loss from severe apical root resorption after orthodontic treatment unless the tooth experienced some other form of trauma.

### 4.6.3 Biological nature of repair

The repair material in humans is principally cellular cementum while in animals root resorption is primarily repaired by acellular cementum.\textsuperscript{149,150} AEFC is able to repair resorptive defects rapidly in rodents because the cementoblasts have a relatively high level of activity while in humans only the CIFC can fill a resorptive defect in a reasonable period of time.\textsuperscript{151}

Human studies on repair of root resorption defects have found that individual variation is large.\textsuperscript{147} Owman_Moll and Kurol\textsuperscript{147} studied repair of orthodontically induced root resorption in adolescents. Healing cementum was of the cellular type and there were no large differences in the healing potential in the cervical, middle, and apical thirds of the root. A study by Langford and Sims\textsuperscript{139} on root resorption following rapid maxillary expansion found that periodontal fibre bundles were found to insert directly into the repair cellular cementum matrix, irrespective of the site of the lesion of the root. Brice et al\textsuperscript{152} studied orthodontic root resorption and repair in human teeth and found that epithelial cell clusters similar to epithelial rests of Malassez were found in areas of repairing orthodontic root resorption.

Studies by Brudvik and Rygh\textsuperscript{153} on rat teeth found that reparative cementum like material appeared in the periphery of the resorption lacunae. Fibroblast-like cells were observed
close to newly formed cementum. Another rat study by Kurihara and Enlow\textsuperscript{154,155} demonstrated the adhesive periodontal attachment to a resorbed dentine surface.

As outlined above, repair of root resorption craters has been demonstrated extensively from both a clinical and a biological perspective. While it has been consistently shown that repair is initiated following the cessation of orthodontic force, little success has been demonstrated in influencing the amount of repair or the speed at which it occurs. For this reason it is of interest to examine methods of preventing root resorption.

4.7 Medication for prevention of root resorption

Medication has been used in a number of studies in an attempt to reduce root resorption following orthodontic tooth movement.

4.7.1 Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs), are commonly used for the control of pain in orthodontics. It has been demonstrated that the uses of NSAIDs may also affect the sequence of tooth movement by reducing the associated inflammatory and bone resorptive process.\textsuperscript{156} This results in a decrease in tooth movement. It therefore comes as no surprise that the alteration in the bone resorptive process and tooth movement is translated into a decrease in root resorption. This has been confirmed in a recent publication on the NSAID nabumetone which showed that there was a reduction in the
amount of root resorption however surprisingly there was no reported effect on the pace of tooth movement.\textsuperscript{157}

### 4.7.2 Corticosteroids

Long term side effects of steroid therapy include disturbances in mineralized tissue metabolism and wound healing, discrepancies in chondrogenesis and osteogenesis, bone loss and osteoporosis.\textsuperscript{158} It therefore follows that chronic corticosteroid use has the potential to influence orthodontic tooth movement. This has been confirmed in a rat study by Kalia \textit{et al}.\textsuperscript{159} who demonstrated that force application resulted in a significant increase in the relative extension of root resorption and formation in rats who received both chronic and acute corticosteroid treatment.

### 4.7.3 Tetracyclines (Doxycycline)

Mavragani \textit{et al}.\textsuperscript{160} studied the effect of low-dose systemic administration of the tetracycline, doxycycline (DC) on orthodontic root resorption and orthodontic tooth movement in rats. The results revealed a significant reduction in root resorption for the DC-administered group although the orthodontic tooth movement did not differ between groups.\textsuperscript{160} The anti-resorptive properties of dxycyclines (DCs) have been attributed to their inhibition of several matrix metalloproteinases (MMPs). MMPs are largely responsible for degrading constituents of connective tissues, not only during pathological tissue breakdown, but also during normal remodeling. Tetracyclines have also been shown to down-regulate the expression of pro-inflammatory and autoimmune mediators,
such as interleukin-1, tumour necrosis factor, nitric oxide, phospholipase A2 and arachidonic acid metabolism.\textsuperscript{160} The role of these mediators in connective tissue breakdown following orthodontic force application has been demonstrated.\textsuperscript{161,162} The reduction in root and bone resorption in the doxycycline group which received orthodontic force may also be attributed to a reduced resorption capacity of the individual clast cell.\textsuperscript{160} It has been shown that tetracyclines can affect several parameters of osteoclast function, such as diminishing the secretion of lysosomal enzymes.\textsuperscript{163}

### 4.7.4 Prostaglandins

Brudvik and Rygh\textsuperscript{164} studied root resorption after local injection of prostaglandin E\textsubscript{2} during experimental tooth movement in a sample of Wistar rats. They found that there was no significant difference in root resorption between the experimentally moved teeth with and without local injection of PGE\textsubscript{2}. Similar results were recorded by Sekhavat and colleagues\textsuperscript{165} who used the prostaglandin E\textsubscript{1}, misoprostol, in a sample of rats. While misoprostol was shown to increase orthodontic tooth movement there was only a minimal effect on the levels of root resorption with a trend toward more root resorption.\textsuperscript{165}

### 4.7.5 Echistatin and RGD peptides

Local injections of echistatin and arginine-glycine-aspartic acid (RGD) have been trialed in rats as a method of preventing tooth movement and therefore enhancing anchorage. These agents are known to inhibit integrin and thus perturb bone remodeling and reduce tooth movement at a local level.\textsuperscript{166} Again it follows that a reduction of tooth movement
and bone remodeling has the potential decrease root resorption following orthodontic
tooth movement and this has recently be confirmed in an experiment on rats after local
administration of echistatin.\textsuperscript{167}

\subsection*{4.7.6 Bisphosphonates}

Liu \textit{et al}\textsuperscript{168} studied the effects of local administration of clodronate on orthodontic tooth
movement and root resorption in rats. Clodronate is a bisphosphonate which strongly
inhibits bone resorption and has anti-inflammatory properties. In their experiment, wistar
rats were injected locally with clodronate and their teeth were moved buccally. They
found that there was a dose-dependent reduction in tooth movement in the rats. Local
clodronate also inhibited root resorption incident to tooth movement.

\subsection*{4.7.7 Fluoride}

Foo \textit{et al}\textsuperscript{169} studied the effect of systemic fluoride intake on root resorption in rats. In
their experiment Wistar rats were fed fluoridated water at 100ppm. Mandibular molars
were moved mesially using NiTi closing coils. Fluoride was found to reduce the size of
the resorption craters, but the effect is variable and not statistically significant.

\subsection*{4.7.8 Calcium}

Seifi \textit{et al}\textsuperscript{170} assessed the effect of Prostaglandins in association with Calcium Gluconate
on root resorption in rats. Once again they found that there was an increase in OTM
following injections of PGE and a trend towards an increase in root resorption. It is of interest to note that in the group which also received calcium there was a trend toward a decrease in root resorption.

Due to the importance of calcium in the maintenance of healthy tooth structure it is of interest to examine ways of maintaining a favourable balance of minerals in order to protect teeth against OIIRR.
5. Calcium Metabolism in the Dental tissues

As outlined in the early discussion, orthodontic tooth movement involves a substantial manipulation of bone in order to achieve gradual tooth movement. It is therefore clear that interventions involving manipulation of bone should only be carried out when the patient is in stable or positive calcium balance.\textsuperscript{171}

An example of the importance of calcium in the mineralization of the dentoalveolar structures is the relationship between osteoporosis and periodontal disease. Research has demonstrated that dentate women who have pre-menopausal plasma oestrogen levels have less alveolar bone loss than dentate women who were oestrogen deficient.\textsuperscript{171} The question is therefore raised as to whether interventions that reduce bone loss generally will also reduce damage to the hard tissues supporting the teeth.\textsuperscript{171}

5.1 Calcium homeostasis

Bones are the primary source of supply of calcium ions when they are needed to maintain the plasma calcium concentration. 99\% of body calcium is stored in the bones.\textsuperscript{171} Powerful feedback control mechanisms normally maintain the plasma within narrow limits, and these mechanisms have important consequences for the integrity of bones.

Calcium and bone metabolism is controlled by the endocrine system. Plasma calcium concentration is primarily controlled by three hormones which are parathyroid hormone (PTH), 1,25 dihydroxyvitamin D\textsubscript{3} (1,25 (OH\textsubscript{2}) D\textsubscript{3}) and calcitonin.\textsuperscript{62} The major target
tissues for these hormones are the bones, the gastrointestinal tract and the kidney. All three hormones operate in concert to maintain the constancy of the calcium level in body fluids. Other internal factors which affect bone mineral metabolism are insulin, growth hormone, thyroid hormone, phosphate regulating hormone, glucocorticoids, adrenal corticosteroids, sex hormones and vitamins A and C.

5.1.2 Parathyroid hormone

PTH regulates calcium balance by stimulating the kidney to increase the reabsorption of calcium in the distal tubule and to inhibit the reabsorption of phosphate ions in the proximal tubule. Both of these actions increase the plasma calcium concentration.

PTH also exerts some control over the absorption of calcium by the small intestine. This is achieved by stimulating the kidney to increase the rate of activation of vitamin D.

Goldie and King’s study on calcium deficient, lactating rats demonstrated that these conditions resulted in decreased bone density which is consistent with increased parathyroid hormone.63

5.1.3 Dihydroxyvitamin D₃

1,25 (OH₂) D₃ is actually a hormone rather than a vitamin. Adequate amounts of 1,25 (OH₂) D₃ are essential to maintain appropriate plasma calcium and adequate mineralization of bone matrix. The plasma concentration of 1,25 (OH₂) D₃ is regulated
by the kidney, which senses the plasma calcium and phosphate and adjusts the output of 
the hormone accordingly by modifying the rate at which its precursor is hydroxylated. 
1,25 (OH$_2$) D$_3$ acts on the intestine to increase absorption of calcium and phosphate ions. 
This has the effect of making more calcium available for the formation of new bone by 
osteoblasts, thereby preventing a net loss of bone.

1,25 (OH$_2$) D$_3$ acts on the bones by augmenting the action of PTH to resorb bone and 
release calcium into the plasma. It also acts on the kidney by promoting calcium 
retention.

A deficiency in 1,25 (OH$_2$) D$_3$ results in a decreased mineralization of bone. However 
this hormone does not directly induce bone formation.

5.1.4 Calcitonin

Calcitonin is involved with the balance of formation and resorption of bone. The action 
of calcitonin is therefore particularly significant during growth and it appears to be 
relatively unimportant in the mature skeleton. Calcitonin is a highly potent inhibitor of 
bone resorption as it prevents osteoclasts from resorbing bone and inhibiting the 
differentiation of new osteoclasts. It is not thought to play a major role in maintaining 
plasma calcium concentration. It appears to be more related to protecting against 
excessive bone resorption in growing children and possibly during pregnancy.
Due to the importance of these hormones in maintaining the constancy of the calcium level in body fluids it is not surprising that altered endocrine conditions such as hyperparathyroidism\textsuperscript{172,173}, Paget’s disease\textsuperscript{174}, hypophosphataemia\textsuperscript{175}, hypothyroidism, hypopituitarism and hyperpituitarism\textsuperscript{176,177} are associated with altered resistance to root resorption.

5.2 Calcium and Phosphate Metabolism and the skeleton

Three sources of calcium; calcium carbonate (CaCO\textsubscript{3}), calcium citromalate (CCM) and milk have been extensively studied and have all shown to ensure efficient absorption of calcium over a long term (one to four years).\textsuperscript{178} Prince \textit{et al}\textsuperscript{179} demonstrate this in a study on menopausal women, in whom calcium supplements, given as CaCO\textsubscript{3} tablets or as milk, reduce bone loss measured over a two year period.

Phosphate is also important in bone mineralisation and calcium metabolism.\textsuperscript{180} It has been shown that phosphorous must be present for the production of hydroxyapatite.\textsuperscript{178} The dissociation of calcium intake from that of phosphorous may restrict bone metabolism.\textsuperscript{178} This may occur in situations where the calcium source is not ingested with the meal and/or this source contains no Phosphate.\textsuperscript{178}
5.3 The role of calcium in teeth and tooth roots.

The influence of diet on the structure of teeth has been studied in depth. Of particular interest to this study are the factors which influence the hardness of the teeth. Some of the earliest studies in this field were conducted by Mellanby.\textsuperscript{181,182} Mellanby concluded that a low dietary calcium content may result in teeth being soft to the point that they can be cut with a scalpel.\textsuperscript{181} Mellanby demonstrated that well and badly calcified teeth can be produced at will by altering in the diet the relative amounts of calcifying vitamin, found in milk, egg-yolk, cod-liver oil, etc., and anti-calcifying substances found chiefly in cereals.\textsuperscript{182}

A study by Bielacyc and Golebiewska in 1997 demonstrated the importance of calcium and vitamin D in roots of teeth in rats.\textsuperscript{183} Scanning microscope observations showed the increased cementolysis and decreased mineralization of cementum and dentin in rats fed a low calcium and vitamin D-deficient diet. This demonstrated that the hard tissues of the teeth, besides bony system, are also involved in calcium homeostasis.

Of particular interest to dentists is the potential for teeth and tooth roots to remineralize. A study by Clarkson et al in 1991 demonstrated the remineralizing potential of human tooth root organic matricies which did or did not contain soluble non-collagenous proteins including phosphoprotein.\textsuperscript{184} This research suggested that the removal of soluble, noncollagenous proteins, especially phosphoprotein from root caries lesions, may enhance their remineralization potential.\textsuperscript{184}
With respect to root resorption Rex et al\textsuperscript{185} performed an electron probe microranalysis (EPMA) on the concentrations of calcium (Ca), phosphorous (P), and fluoride (F) concentrations in human first premolar cementum after the application of light and heavy orthodontic forces. The application of heavy forces caused a significant decrease in the Ca concentration of cementum at certain areas of periodontal ligament tension which demonstrates the demineralising nature of orthodontic force.

Seifi et al\textsuperscript{170} demonstrated how the provision of calcium can potentially protect against root resorption. They studied the effect of submucosal injections of Prostaglandins in association with intraperitoneal injections of Calcium Gluconate on root resorption in rats and demonstrated that this combination demonstrated a trend toward a decrease in root resorption.\textsuperscript{170} They postulated that this may be a result of the transient hypoparathyroidism and diminished resorptive activity subsequent to injection of the calcium compound. The association of root resorption with hypothyroidism has been described previously by Newman.\textsuperscript{78} Seifi et al\textsuperscript{170} proposed that it seemed likely that raised serum calcium levels may inhibit PTH secretion and therefore inhibit root resorption.

These studies demonstrate the importance of maintaining adequate mineralisation of the dental tissues in order prevent the breakdown of the root structures. As Brudvik and Rygh\textsuperscript{142} have demonstrated, root resorption involves the removal of mineralized cementum. It would therefore follow that if we can maintain adequate mineralization of
the dental tissues then there may be a possibility to protect teeth against orthodontic root resorption

### 5.4 Promoting calcium availability

The NIH (USA) recommends that humans should consume between 800mg to 1500mg of calcium per day depending on age and gender.\textsuperscript{186} The US department of Agriculture cites figures showing that only about 13% of girls and 36 percent of boys aged 12-19 in the United States consume at least the amount of calcium recommended by the NIH(USA).\textsuperscript{187} This is of concern because nearly 90% of adult bone mass is established before the age of 20.\textsuperscript{171}

Dairy products are particularly effective in storing calcium because the milk protein casein stabilizes the structure of the liquid which allows it to maintain its high calcium phosphate concentration without allowing precipitation.\textsuperscript{188} It follows that the provision of dietary casein would be of potential benefit in making calcium available for the protection of tooth roots.
6. Casein Phosphopeptides

6.1 Definition

Casein Phosphopeptides (CPP) are multi-phosphorylated peptides from an enzymatic digest of the bovine milk protein casein. In milk, casein stabilizes the structure of the liquid in order for it to maintain its high calcium phosphate concentration without allowing precipitation.

6.2 CPP and medicine

Bioactivity of phosphopeptides yielded after tryptic hydrolysis of casein (CPP) was reported more than 50 years ago by Mellander who discovered that CPP were found to improve calcium balance in rachitic newborns. Mellander demonstrated that calcium bound to phosphopeptides could be absorbed from the digestive tract and promote bone calcification in rachitic children.

CPPs have been shown to enhance calcium absorption by increasing calcium solubility in vitro and in situ. It has been demonstrated that CPPs enhance paracellular transport of calcium in the distal small intestine in elderly female rats. This has been confirmed by Saito and colleagues who found that CPP supplementation has the effect of significantly increasing intestinal Ca absorption, at least under conditions of marginal dietary Ca levels.
The positive effects of milk casein phosphopeptides on bone retention has been demonstrated in a number of rat models. Tsuchita et al\textsuperscript{180} demonstrated in an \textit{in vitro} study that there was an increase in absorption of calcium and bone retention in rats. They demonstrated that Casein Phosphopeptides have an inhibitory effect on bone loss in aged ovariectomized rats which could be due to their effects on phosphorous and calcium metabolism.\textsuperscript{180} It was found that rats fed a CPP diet had an increase in urinary calcium excretion and a decrease in urinary phosphorous excretion. This study showed less bone loss in rats fed CPP than in rats given Ca and P as pure minerals.\textsuperscript{180} Ma and Yamaguchi\textsuperscript{192} demonstrated similar positive effects associated with CPP in a study that showed a combination of CPP and genistein had a synergistic-anabolic effect on bone components in rats with increasing age. They postulate that this combination may have a role in the prevention of osteoporosis.

The positive effects of CPP supplementation on the skeleton has also been demonstrated in humans. Heaney \textit{et al}\textsuperscript{194} studied the effects of administering 87.5mg of CPP to 35 normal post-menopausal women as a part of a standard test meal containing a calcium load of 250mg. Their findings suggested that caseinphosphopeptide supplementation is particularly useful, for persons with low basal absorptive performance.

The positive effect of CPP on the mineralization of the skeleton has been demonstrated in both human and animal studies. Like bone, teeth also require a balance in mineral content in order to prevent resorption and as such the question is raised as to whether or not CPP can offer potential benefits to teeth which are at risk of losing mineral content.
6.3 CPP and Dentistry

In recent years there has been significant interest in the potential for casein phosphopeptides to prevent and remineralise carious lesions. Dental caries is a saliva and carbohydrate modified bacterial disease. The bacteria involved in the disease produce acid as a metabolic byproduct which causes demineralization of the dental tissues which often leads to cavitation of the tooth.

The use of a remineralizing solution containing calcium and phosphate ions has not been clinically successful due to the low solubility of calcium and phosphates. Calcium and phosphate ions at low concentrations do not incorporate to any significant degree into plaque or localize at the tooth surface.

In contrast, dairy products are a food group which are widely recognized to exhibit anticaries activity. Animal and in vivo caries models have demonstrated that the components responsible for the anticariogenic nature of dairy products are the casein, calcium and phosphate. A study by Reynolds et al in 1995 on rats demonstrated that the tryptic digest of casein with the phosphopeptides selectively removed showed no anticariogenic activity. On the other hand, the synthetic octapeptide-calcium phosphate complex significantly reduced caries activity which confirmed that this calcium-phosphate-stabilizing portion of the casein phosphopeptides is associated with anticariogenicity.
CPPs have the ability to stabilize calcium phosphate in solution and substantially increase the level of calcium phosphate in dental plaque.\textsuperscript{199,200} Appendix 2 Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes have been shown to localize at the tooth surface and prevent caries in laboratory, animal and human in situ caries models.\textsuperscript{189,198} Reynolds has proposed that the anticariogenic mechanism for CPP-ACP is the localization of ACP at the tooth surface which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization.\textsuperscript{189} Roberts et al have also demonstrated that this rematerialising capacity is effective in reducing the whiteness of fluorotic lesions.\textsuperscript{201}

Furthermore it has also been demonstrated that the incorporation of CPP into salivary pellicles reduced adherence of both \textit{S.sobrinus} and \textit{S.mutans} significantly and thus has bacteriostatic/bacteriocidal effects.\textsuperscript{188,202} Rose demonstrated the mechanism for this as being the competition for binding sites on \textit{S.mutans} between CPP-ACP and calcium.\textsuperscript{188}

### 6.4 Delivery of CPP in Rats

Anticariogenicity of CPP-ACP has been investigated using specific-pathogen-free rats orally infected with \textit{Streptococcus sobrinus}.\textsuperscript{198,189} The rats had the CPP-ACP solution exposed to their teeth through their drinking water. The study demonstrated a significant reduction in caries activity with 0.1% w/v CPP-ACP producing a 14% reduction and 1.0% w/v CPP-ACP a 55% reduction relative to the distilled water control.\textsuperscript{189}
6.5 Delivery of CPP in humans

Reynolds\textsuperscript{203} demonstrated in a human intra-oral caries model that when caseinate was digested with trypsin, the tryptic peptides of casein were found incorporated into an intra-oral appliance plaque and this was associated with a substantial increase in the plaque’s content of calcium and phosphate. The tryptic peptides responsible for the anticariogenic activity were concluded to be the calcium phosphate sequestering phosphopeptides.\textsuperscript{189}

It has been demonstrated in a human model that two exposures per day of the CPP-ACP solution produced a 19\% reduction in enamel mineral loss relative to the control enamel.\textsuperscript{189} CPP was incorporated into the plaque resulting in a 2.4 fold increase in the plaque calcium and a 2.6 fold increase in plaque inorganic phosphate.\textsuperscript{189}

CPP-ACP has also been shown to remineralize enamel subsurface lesions in situ when delivered in a sugar-free gum.\textsuperscript{204,205} The results showed that sugar-free gum containing CPP-ACP is superior to an equivalent gum not containing CPP-ACP.\textsuperscript{204,205} CPP-ACP has also been incorporated into a self-cured glass-ionomer cement (GIC).\textsuperscript{206} Incorporation of 1.56\% w/w CPP-ACP into the GIC significantly enhanced the release of calcium, phosphate, and fluoride ions at neutral and acidic pH.\textsuperscript{206} The release of CPP-ACP and fluoride from the CPP-ACP containing GIC was associated with enhanced protection of the adjacent dentin during acid challenge \textit{in vitro}.\textsuperscript{206}
6.6 CPP and root resorption

The ability for CPP to effectively provide bioavailable calcium for maintaining mineralization of both the skeleton and the dental tissues has been extensively demonstrated in both rat and human models. Like bone, teeth also require a balance in mineral content in order to prevent resorption and as such the question is raised as to whether or not CPP can offer potential benefits to teeth which are at risk of losing mineral content. Furthermore CPP has been demonstrated to be a safe, natural substance when delivered topically or systemically in both rat and human models. It therefore follows that CPP could potentially be an effective and safe way of preventing root resorption.
7. Research Tools and Methodologies

7.1 Subject

Rats have proved to be an appropriate subject for tooth movement and root resorption experiments on numerous occasions. 57% of animal studies on orthodontic tooth movement used rats during the years of 1981 to 2002. Furthermore rats have been used as experimental subjects when studying the effectiveness of CPP in both anticariogenic experiments and nutritional experiments.

7.1.2 Appliance Design

Wistar rats have been commonly used as the subjects in animal experiments involving tooth movement. The majority of experiments have moved maxillary molars mesially. Appendix 3 It is appropriate to move the molars mesially because the buccal bone in rats is very thin and also more compact than the bone on the mesial side which means that bucco-lingual movement is contraindicated.

In order to study tooth movement in rats it is important to design a reliable and effective appliance. A review of the literature by Ren et al revealed that a quarter of all studies between 1981 and 2002 used elastics to produce orthodontic tooth movement. The use of elastics has the significant disadvantage of force decay. Nickel Titanium coils offer a force which is more continuous in nature and are thus more appropriate for use in an experimental tooth movement appliance with the view of analyzing root resorption.
Ren et al\textsuperscript{207} designed a tooth movement appliance based on an extensive review of the literature. Their appliance used sentalloy closed coil springs which were tied from the maxillary incisors to all three maxillary molars. The study was a split-mouth design with the experimental side randomly chosen and the contralateral side used as the control. Other studies have used similar designs but have only tied the coils from the incisors to the first molar.\textsuperscript{164,170}

### 7.1.3 Measurement of tooth movement

There has been some difficulty in measuring tooth movement in rats due to continued eruption of the incisors and possible distal tipping of the incisors used as anchorage. In the experimental design proposed by Ren et al\textsuperscript{207} tooth movement was determined by measuring the incisor to molar distance before and after appliance activation. Their measurements were taken between the most mesial point of the maxillary molar unit and the enamel-cementum border of the ipsilateral maxillary incisor at the gingival level. X-Ray photographs of the appliances used by Ren et al\textsuperscript{207} demonstrated that continuous eruption of the upper incisors was effectively prevented and that abrasion of the maxillary incisors was compensated by overeruption of the mandibular incisors.

Intraoral measurement of incisor to molar distances in rats under anaesthetic is difficult. In an experiment which only applies force to one molar, an alternative method for measuring tooth movement is to measure the distance at the level of the contact point between the tooth to which the force has been applied and the adjacent molar. This
method assumes that the teeth were in contact before the appliances were inserted and that physiologic movement of the adjacent tooth was negligible. The advantage of this method is that it is not necessary to take intraoral measurements at the time of appliance insertion. King and Fischlschweiger\textsuperscript{208} measured this distance using calibrated thickness gauges between the maxillary first and second molars at sacrifice. This gap can also be measured very accurately using digital measurement tools on micro CT images.

### 7.2 Analysis of root-resorption

Root resorption has been studied and measured by a variety of methods which include histology, light microscopy, electron microscopy and radiography.

#### 7.2.1 Histology

While clinical studies and analyses of plain radiography reveal a varied incidence in root resorption, histological studies were able to demonstrate that root resorption of varying degrees is a common side effect of orthodontic tooth movement.\textsuperscript{83}

#### 7.2.2 Light Microscopy

Light microscopy allows another view of resorption craters.\textsuperscript{97} Appendix 4 Light microscopy has also been used to quantitatively analyse root resorption craters. Owman-Moll and Kurol\textsuperscript{147} used light microscopy to register root resorption and repair on one randomly chosen histological section from each of three different sectional levels on a
tooth. Surface extension and depth of each resorption lacuna was measured to the nearest unit. This technique has been criticized because it was felt that this technique could not account for the variation in size, shape and depth of the craters and variation in root morphology and consequently accurate quantitative measurements of these craters could not be achieved.\textsuperscript{209}

Brudvik and Rygh\textsuperscript{153} used light microscopy to study the initial attack and the resorption-repair sequence on root surfaces exposed to compression. They used light microscopy to measure the total length of the resorption lacunae in a coronal-apical direction.

Igarahi \textit{et al}\textsuperscript{210} have analysed root resorption craters by feeding microscopic images to a television monitor with a CCD video camera. The data is expressed as square micrometers

While light microscopy provides a useful tool for analysing craters, the two dimensional images do not allow an accurate quantitative analysis of root resorption.

\subsection*{7.2.3 Transmission Electron Microscopy}

While it is possible to analyse root resorption craters using TEM sections it is difficult to get a quantitative analysis using this tool because the shape, size and location of the craters means that it is likely that some may be lost during sectioning. While TEM images provide useful 2D images they are not designed to easily produce 3D reconstruction of specimens.
7.2.4 Scanning Electron Microscopy

SEM provides an overall image of resorption craters on the root surface with minimal tissue preparation. SEM images have been used to assess root resorption craters in 2 dimensions. King and Fischlshweiger\textsuperscript{208} used point counting volumetry to quantitatively assess root resorption. In this technique micrographs were placed under a regular grid lattice, and intersections of the grid coincident with a resorption lacuna were scored. Other studies by Barber and Sims\textsuperscript{148} used a Zeiss MOP digitizer to calculate the resorption-affected areas in buccal profile from photographs of areas of resorption. There is a risk of parallax measurement errors in 3D when using this technique due to the curved nature of root resorption craters.\textsuperscript{209} Furthermore, because the composite micrographs are pieced together, there is a risk of error if the craters are along the edges of the micrograph.\textsuperscript{209}

The problems of parallax error with 3D volumetric measurements using SEM images can be resolved by recording stereo images of the craters and converting this into an 8-bit grayscale depth map.\textsuperscript{211} This method has been shown to be highly accurate and reproducible.\textsuperscript{209} However this method is time consuming and difficult to perform for large samples.
7.2.5 Radiography

Wilhelm Conrad Roentgen was the first to study the x-ray phenomenon which occurs when an electric current passes through a gas of extremely low temperature. The science and technology of x-rays has since progressed to allow the visualisation of both living and inanimate objects from a variety of perspectives.

7.2.5.1 Plain Radiographs

Plain radiographs such as periapical radiographs, dental panoramic films and lateral cephalograms are the most popular views in dentistry. However these images have the inherent problems associated with 2D representations of 3D objects. The magnification and parallax errors associated with plain radiographs render these images of use only for qualitative descriptions of root resorption.

There have been attempts to reproduce three dimensional structures from a two dimensional film. These techniques are known as laminography or focal plane tomography. It involves translating an object/subject together with the detecting medium in such a way that only one narrow slice parallel to the translation plane is in focus. Sharp images are difficult to obtain because of the thickness of each slice and the smearing of images of the plane outside the imaging plane across the image of the plane of interest.
7.2.5.2 Computer Tomography

With the advent of digital computers, an approach called computer tomography became possible. Early in the 1970s Hounsfield\textsuperscript{214} developed a commercial system for medical imaging. It can be difficult keeping the dose of x-rays to a minimum and the duration must be limited as involuntary movement by the subject will cause distortions in the image. However these considerations need not apply to imaging of inanimate objects.

Three dimensional images are achieved by taking a series of x-ray projections through the slice at various angles around an axis perpendicular to the slice. From this set of projections, the x-ray absorption map is computed, and by taking a number of slices, a three dimensional map is produced.

The resolution of a two dimensional image is given in terms of pixels which are a two dimensional representation of the smallest unit of colour value within an image. This colour value can be a shade of grey or a colour. Imaging software assigns the dimensions of a pixel (x and y axis). The number of pixels per surface area gives the resolution of the image. The quality of an image improves when there are more pixels per unit area which is described as a higher resolution. Pixels are no longer valid when considering three dimensional images. The term voxel is used in three dimensional imaging. Voxels include the dimension of depth in order to represent a volumetric value in space.
7.2.5.3 Micro Computer tomography

Micro computer tomography has been developed to enable higher resolutions of smaller samples. Current technology limits the use of micro computer tomography to small, inanimate specimens, such as bone and extracted teeth samples. Micro computer tomography can be taken for investigations without damaging or removing any material. This system is therefore useful for viewing biological structural materials such as calcified tissues.

The SkyScan 1172 is a micro CT scanner which has been used extensively in medical research. It is a compact desktop system for microscopy and micro tomography. It consists of an x-ray shadow microscopic system and a computer with tomographic reconstruction software. This scanner is a cone beam x-ray source with a spatial resolution of between 2 and 5 micrometres. The recommended sample size is a diameter of 1.5 cm and a height of 3 cm. The sample is placed on a rotating platform, and depending on its proximity to the x-ray beam, there is a magnification factor. A higher magnification gives greater detail. A high resolution Charged Coupled Device with a resolution of 1024 x 1024 pixels will detect the incoming x-rays. The information collected by the SkyScan x-ray micro tomograph is stored and reconstructed to 8 Bit Mapped Picture files.

The SkyScan micro tomograph uses a non destructive procedure to create a three dimensional reconstruction of the objects’ inner structure from two dimensional x-ray shadow projections. The software package, VGStudioMax v1.2 is used to collate all the
axial slices to form a three dimensional reconstruction of the scanned image. This software package also has the function to remove the bony tissue around the scanned tooth, which facilitates the viewing of any defects on the cemental surface. This has advantages for the analysis of rat molars because it eliminates the need for their extraction. There is a significant risk of root fracture and damage to tooth roots when extracting rat molars.

If not conducted properly a quantitative evaluation of root resorption will have an impounding effect on the correlations between the magnitude of force duration and extent of tissue destruction. Three dimensional micro CT imaging facilitates the visualisation of any defects and root resorption craters on the surface of the cementum, indicating a resorptive defect. Craters and defects can then be measured and an accurate volumetric calculation of the defect can then be undertaken. Appendix 5
8. References


129. Reitan K. Effects of force magnitude and direction of tooth movement on different alveolar bone types. Angle Orthodontist. 1964;34:244-255.


201. Roberts MJ ML, Reynolds EC. Remineralisation of fluorotic enamel lesions by casein phosphopeptide-amorphous calcium fluorophosphate (CPP-ACFP) solution.


9. Manuscript
9.1 Abstract
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9.3 Materials and Methods
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9.5 Discussion
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9.7 Acknowledgement
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10. Future Directions

The results of the present study indicated that CPPs seem to have a variable effect on the volumetric quantification of root resorption. While on average, the amount of resorption observed in rats fed dietary CPPs was less, individual variability makes this effect statistically insignificant.

The preventive effects of systemic CPP on orthodontically induced inflammatory root resorption has not been previously investigated. While the amount of dietary CPPs fed to the rats was based on previous research\textsuperscript{192}, it is possible that varying levels of CPP may have varying effects on the amount of root resorption following the application of orthodontic force. Furthermore, it is possible that varying the time length of administering dietary CPPs before appliance placement, may have an effect on the amount of root resorption.

CPPs has been shown to be effective in delivering bioavailable calcium.\textsuperscript{180,194} It may therefore be of interest to confirm this relationship by varying the dietary intake of calcium with dietary CPP and studying the effect that this has on OIIRR.

Although it is possible that CPPs may have a beneficial effect on reducing cementum solubility it may be counteracted by its anabolic effect on bone mass which explains some of the variability observed in this study. The association between the amount of tooth movement, the amount of root resorption and the mineralisation of the alveolar bone therefore warrants further investigation. This may be achieved by measuring the
percent bone ash. Another way of investigating the relationship would be to study root resorption in aged, ovariectomised rats.

Additionally, after two weeks of tooth movement, inactivation of the Niti coil will start the healing process of resorption craters. It would be of interest to investigate the potential for CPP to influence the repair of the resorption craters.

The individual variability observed in the present study also warrants further investigation. It would be of interest to look at the unloaded, contralateral teeth, to see if rats which had large amounts of root resorption on orthodontically loaded teeth also had large amounts of physiologic root resorption on the unloaded teeth. It would also be of interest to determine whether or not rats which showed reduced root resorption also showed reduced tooth movement. This would give further insight into individual susceptibility to root resorption.
11. Appendix

Appendix 1

CPP – ACP complex
Professor Eric Reynolds, University of Melbourne.
Appendix 2

CPP – ACP in Plaque
Professor Eric Reynolds, University of Melbourne.
Appendix 3

Schematic drawing of tooth movement appliance as designed by Ren et al^207

Appendix 4

Dissecting Light Microscope view of the mesial root of the maxillary first molar of a wistar rat. This photograph is of the junction between the porous apex and the cervical third of the root.

Dissecting Light Microscope view of the mesial root of the maxillary first molar of a wistar rat. This photograph is of the porous apex of the root.
## Appendix 5

### Raw Data

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