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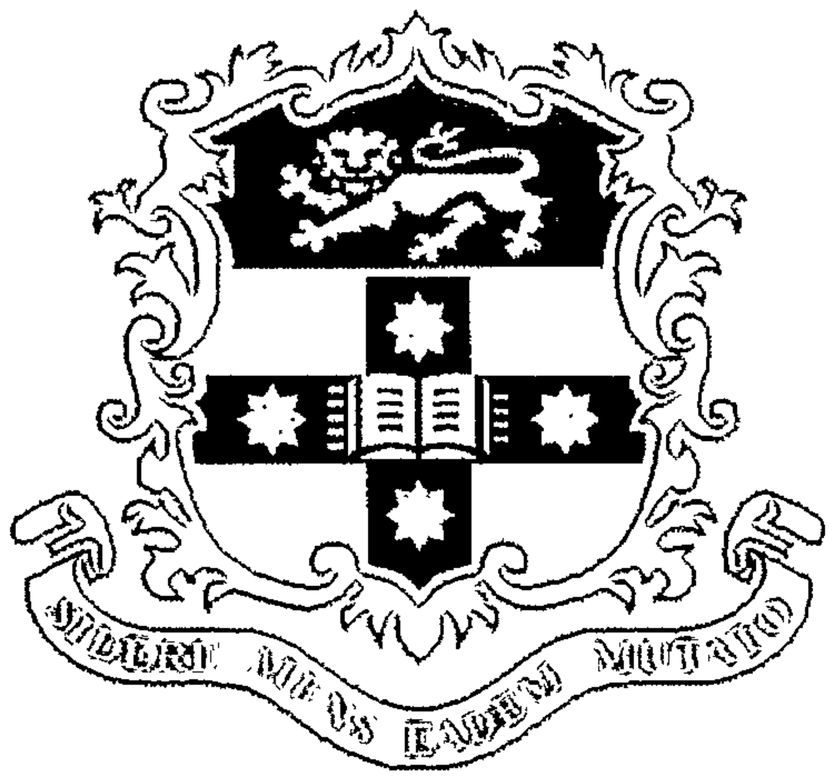

REPAIR OF ROOT RESORPTION FOUR AND EIGHT WEEKS FOLLOWING THE APPLICATION OF CONTINUOUS LIGHT AND HEAVY FORCES FOR FOUR WEEKS: MICRO-CT AND HISTOLOGY STUDIES

Lam Linda CHENG
BDSc(Hons) BDentSt

Discipline of Orthodontics
Faculty of Dentistry
University of Sydney
Australia

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Dedication

To my beloved family, Dad, Mum, Sis and Bro,

For their endless love and support throughout the years of my studies.

For their tolerance and encouragement during troubled times.

My lifetime accomplishments belong to you all.

Thank You. I love you all!

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 has not been submitted to any other University or Institution for a higher degree.

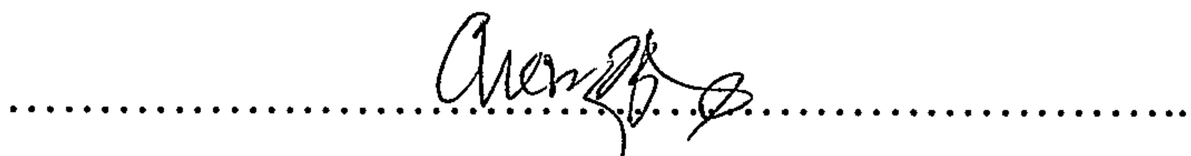

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Table of Contents

Abbreviations

2D	Two dimensional
3D	Three dimensional
AAC	Acellular afibrillar cementum
AEFC	Acellular extrinsic fibre cementum
ANOVA	Analysis of Variance
Ca	Calcium
CAT	Computed axial tomography
CEJ	Cemento-enamel junction
CIFC	Cellular intrinsic fibre cementum
DPT	Dental panoramic tomograph
EARR	External apical root resorption
ECRM	Epithelial cell rests of Malassez
ER	Endoplasmic reticulum
ERR	External root resorption
F	Fluoride
FEM	Finite element model
GCF	Gingival crevicular fluid
H&E	Haematoxylin and Eosin
HEBP	1-hydroxyethyliden-1, 1-bisphosphonate
HERS	Hertwig's epithelial root sheath
IGF(s)	Insulin like growth factor(s)
IL-1β	Interleukin-1 beta
LIPUS	Low-intensity pulsed ultrasound
micro-CT	Micro-computed tomography

NSAIDs	Non-steroidal anti-inflammatory drugs
OIIRR	Orthodontically induced inflammatory root resorption
OPG	Osteoprotegerin
P	Phosphorus
PDL	Periodontal ligament
PGE2	Prostaglandin E2
PTH	Parathyroid hormone
RANKL	Receptor activator of nuclear factor kappa B ligand
rER	Rough endoplasmic reticulum
RME	Rapid maxillary expansion
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TMA	Beta-titanium molybdenum alloy
TNF	Tumour necrosis factor
TRAP	Tartrate resistant acid phosphatase
XMT	X-ray microtomography

1. Introduction

Root resorption has been studied extensively for over a century. It is a physiologic or pathologic process that results in a loss of substance from mineralised cementum and dentine.[1,2] External root resorption (ERR) in orthodontics is a pathologic process that seems to be related to the local injury of the periodontal ligament (PDL) which occurs in association with the removal of hyalinised tissue.[3,4] The contradictory fact is that root resorption is an inflammatory process and this inflammation is required for tooth movement. Therefore, root resorption is commonly known to be an unavoidable adverse effect of orthodontic treatment. Root resorption continues in hyalinised tissue even after active force has been terminated.[5] However, a reparative process in the periodontium commences when the applied orthodontic force is discontinued or reduced below a certain level.[6,7] This healing process can occur as early as the first week of retention following orthodontic treatment and increases over time.[8-10] There are biological and mechanical factors that influence the severity of orthodontic induced inflammatory root resorption (OIIRR). Mechanical causative factors can be controlled by the clinician to minimise the adverse effect of root resorption and allow initiation of repair. The majority of root resorption studies were achieved two dimensionally through radiographs,[11] light microscopy,[12] scanning electron microscopy (SEM)[8] and transmission electron microscopy (TEM).[13] Three dimensional (3D) methods including stereo SEM imaging and X-ray microtomography (XMT) are more accurate and reliable quantitative measuring tools in root resorption studies.[14-16] The objective of this review is to focus on OIIRR and the associated reparative process. Different research tools that are used in root resorption studies will also be reviewed.

2. Cementum

2.1 Definition and Functions of Cementum

Cementum is an avascular, non-uniform, mineralised connective tissue that covers the roots of the teeth which functionally belongs to the periodontium.[17,18] There are little to no knowledge as to its origin, differentiation and cell dynamics during normal development, repair and regeneration. Cementum shares similar physical, chemical and structural features with the compact bone.[19] It is also developmentally controlled by similar factors.[19] In addition, the diseases that alter the properties of bone affect the properties of cementum.[19] For example, Paget's disease results in hypercementosis whereas hypophosphatasia results in no cementum formation.[19] However, cementum differs from bone in that it contains no neural or vascular tissue and continues to grow in thickness throughout life unless disturbed by periapical or periodontal pathology.[17,18]

Cementum contributes two main functions to the dental apparatus.[18] It anchors the principal collagen fibres of the PDL to the root surface. It also inherits a dynamic and highly responsive feature of adaptive and reparative function which is important to maintain the occlusal relationship, the integrity of the root surface and its function in tooth support.

2.2 Types of Cementum

Different types of cementum can be distinguished based on:[17]

1. The presence or absence of enclosed cementocytes within its matrix. Cellular cementum has cementocytes within the lacunae and the absence of cementocytes indicates acellular cementum;
2. The timing of development. Cementum that is formed during the pre-functional stage, i.e. throughout root formation, is the primary cementum which is usually comprised of the acellular extrinsic fibre cementum (AEFC). Cementum that is formed during functional stage which starts when the tooth is in occlusion and continues throughout life is secondary cementum which is usually comprised of the cellular intrinsic fibre cementum (CIFC);
3. The origin of the collagenous fibres of the matrix. The intrinsic fibres originate from cementoblast activity and extrinsic fibres result from the incorporation of the PDL fibres.

Human teeth have three fundamentally different varieties of cementum which usually have tooth type specific distribution patterns.[18] Acellular afibrillar cementum (AAC) covers minor areas of the enamel especially at the cemento-enamel junction (CEJ). AEFC is mainly found on the cervical and middle root portion and also the apical root portion of the front teeth. CIFC usually covers the root surface where no AEFC has been laid down on the dentine which is the furcation areas and on some apical root portions. CIFC is responsible for the repair process of previously resorbed roots. CIFC can overlay AEFC and vice versa to form cellular mixed stratified cementum. This usually occurs in the apical root portions and the furcation areas.

2.2.1 Acellular Afibrillar Cementum[18]

Acellular Afibrillar Cementum (AAC) is a mineralised matrix that is similar to AEFC that has no collagen fibril or embedded cell. The deficiency of the collagen fibril renders this type of cementum to have no function in tooth attachment. Under light microscope, the AAC appears basophilic and rather uniform. However, TEM reveals multiple layers of varying electron density and different texture which can be either granular or reticular.

2.2.2 Acellular Extrinsic Fibre Cementum[18]

The high density of collagen fibres found in the AEFC indicates its function as a tooth anchor to the surrounding bone. It also has the capacity to adapt to functionally dictated alterations such as mesial tooth drift.[20] Under light microscope, AEFC seems relatively structureless. However, under polarised light and with special staining, two sets of striation are revealed. The striations running parallel to the root surface indicate incremental deposition. The short striations at right angles to the root surface indicate the inserted mineralised PDL collagen fibre bundles. Under electron microscope, these collagen bundles can be seen to enter cementum where they become fully mineralised.

2.2.3 Cellular Intrinsic Fibre Cementum

The adaptive behaviour of the CIFC maintains the tooth in its proper position. The capacity of rapid growth allows CIFC to repair a resorptive defect on a root.[21] CIFC is characterised by the entrapment of cementoblasts in the extracellular matrix as cementum is deposited.

2.3 Composition of Cementum

Cementum is composed of 45 per cent inorganic material, 33 per cent organic matrix and 22 per cent water.[22] The relative amount of mineral component varies depending on the cementum type, location and root resorption activity.

2.3.1 Organic Matrix

The organic matrix of the cementum consist of approximately 90 per cent type I collagen and five per cent type III collagen.[17,23] Type I collagen is found in CIFC which accommodates mineral deposition in the early stages of cementogenesis, development and repair. Type III collagen is found in high concentrations during development and repair and regeneration of mineralised tissues. Low levels of type XII collagen can be detected in cementum and are more abundant in the PDL. Type V, and type XIV are occasionally found in the extracts of mature cementum and may be considered as contaminants from the PDL region. They are produced by the PDL fibroblasts associated with collagen fibres inserted into the cementum.[17] The collagen in mineralised tissues plays important structural and morphogenic roles. In addition, it provides a scaffold for the mineral crystals.[24]

2.3.2 Non-collagenous Proteins

There are numerous non-collagenous proteins found in the mature cementum. These include bone sialoprotein, osteopontin, vitronectin, fibronectin, osteocalcin, γ-carboxyglutaminic acid, osteonectin, proteoglycans, several growth factors and two adhesion molecules.[18] Their function in cementum is to promote cell attachment, migration and stimulate protein synthesis of gingival fibroblasts and PDL cells.

2.3.3 Mineral Component

Similar to any calcified tissues, the mineral component of cementum is composed of hydroxyapatite with small amounts of amorphous calcium phosphates.[18] The mineral crystals are arranged with their crystallographic c-axis parallel to the long axis of the collagen fibril with which they are associated with.[25] The crystallinity of the mineral component in cementum is lower than other hard tissues which leads to greater capacity for absorption of fluoride and other elements but readily decalcifies in the presence of acidic conditions.[18] The cementum also contains other elements that have been absorbed from the tissue fluid during initial crystallisation.[18] These include 0.5 to 0.9 per cent magnesium, 0.9 per cent fluoride and 0.1 to 0.3 per cent sulphur.[18] Other trace elements that may be present are copper, zinc and sodium.[18]

Generally, AEFC appears more highly mineralised than CIFC and cellular mixed stratified cementum.[26] The difference can be explained by the presence of uncalcified spaces such as lacunae and the uncalcified core of Sharpey's fibres. In addition, the matrix of AEFC may be more completely mineralised because its formation is a slow process that allows longer and direct contact of tissue fluids.[26]

2.4 Development of Cementum

Cementum is secreted by cementoblasts onto the surface of root dentine or in some animals onto the crown enamel.[27] Its formation begins following the onset of root formation and elongation. Hertwig's epithelial root sheath (HERS) guides the formation of the root. The HERS is a collar of epithelial cells resulting from the apical elongation of the enamel organ.[17] Prior to cementogenesis, the HERS disintegrates and dental follicle cells penetrate the epithelial layer to access the root surface. Cementoblasts deposit the cementum and constitute a large number of these invading dental follicle cells.[27]

Slow forming cementum is usually acellular whereas in rapid forming cementum, cementoblasts may be trapped in the lacunae in the cementum. This type of cementum is cellular. Both types of cementum are formed in layers throughout life in response to the physiological demands on the tooth. The production of cementum continues throughout life unless upset by periodontal pathology.[18] A linear relationship between the depth of the cementum and the age of the tooth has been found with an average thickness of 0.095mm at the age of 20 years and 0.215mm at the age of 60 years.[28]

3. Root Resorption

3.1 Definition and Classification of Root Resorption

Root resorption is a physiologic or pathologic process that results in a loss of substance from dentine or cementum.[1] Physiological root resorption occurs during the exfoliation of deciduous teeth and involves internal and external resorption processes. Pathological root resorption may either be internal or external in origin. Internal resorption is initiated from within the pulp and external resorption arises from the periodontium affecting the external and lateral surface of the tooth. Internal resorption may be classified as internal replacement resorption or internal inflammatory resorption. To this simple classification may be added internal tunnelling resorption. There are several classifications of ERR. Andreasen[29] has classified ERR following trauma to a tooth as surface resorption, external inflammatory resorption and replacement resorption. Tronstad[30] further differentiated external inflammatory resorption into transient inflammatory resorption and progressive inflammatory resorption. Tronstad[30] also added cervical resorption to the classification. Brezniak and Wasserstein[31] expanded the classification by including orthodontically induced inflammatory root resorption (OIIRR) for ERR that is caused by orthodontic force.

4. Orthodontically Induced Inflammatory Root Resorption

When an orthodontic force is applied to a tooth, an inflammatory process is initiated to resorb bone on the pressure side and deposit bone on the tension side which results in tooth movement. Similarly, OIIRR, as its name suggests, is a result of this inflammatory phenomenon that occurs on the cemental surface of the tooth root. Cementum is generally more resistant to resorption when compared to bone but resorption of cementum and dentine can still occur during this inflammatory process.

4.1 History of Orthodontically Induced Inflammatory Root Resorption

Root resorption of permanent teeth was first discussed by Bates in 1856 as a process referred to as "absorption".[32] Bates stated the cause to be the traumatisation of periodontal membrane. In 1914, Ottolengui[33] identified root resorption that was specifically caused by orthodontic treatment. Later, Ketcham[34] reported apical root resorption of permanent teeth through a radiographic study. Root shortening could be an anatomic variation and resorption could be a result of impaction or orthodontic treatment. In that study, Ketcham concluded that maxillary anterior teeth were more prone to root resorption than mandibular anterior teeth. The study suggested that pre-treatment and in-treatment radiograph was essential to monitor the extent of root resorption. Ketcham,[35] later, suggested that hormonal imbalance and dietary deficiencies were possible reasons for the occurrence of root resorption. During that era, both absorption and resorption were used to describe the loss of apical root material. It was not until 1932, that Becks and Marshall[36] suggested the use of

resorption instead of absorption because "in all cases in which formed tissues are destroyed and taken up by the blood or lymph stream, one should, in medical or dental literature, speak only of resorption".

4.2 Incidence of Orthodontically Induced Inflammatory Root Resorption

Incidence of OIIRR was commonly determined by radiographic examination. Early studies on the incidence of root resorption had inherent flaws as standardisation of radiograph had not been introduced. In addition, plain film radiographs were two dimensional (2D) representation of a 3D tooth. This means that resorption craters located on the buccal and lingual surfaces of the tooth cannot be detected on conventional periapical radiographs.[37] Histological studies reported a greater than 90 per cent occurrence of OIIRR.[38,39] Therefore, it could be inferred that root resorption caused by orthodontic tooth movement was much more prevalent than what had been documented.

In 1927, Ketcham[34] identified 21 per cent of the 500 patients he surveyed radiographically had distinct evidence of root resorption after orthodontic treatment. Root resorption also occurred in one per cent of the people who were not subjected to orthodontic therapy.[34] Almost a decade later, Rudolph[40] reported a high OIIRR incidence of 75 per cent. In a later study, he found five per cent of the control group and 100 per cent of the study group displayed root resorption.[41] Becks[42] found 32 per cent had root resorption prior to orthodontic treatment and the frequency seemed to increase to 73.6 per cent after orthodontic treatment. Becks[42] concluded from the study that root resorption was not solely attributed to orthodontic

treatment. In 1951, Henry and Weinmann[43] analysed the number, size and distribution of the physiological resorption found on cementum. They revealed 90.5 per cent of the teeth examined displayed areas of resorption with the greatest number of resorption at the apex of the root. Similarly, Massler and Malone[44] found 86.7 per cent of patients who had evidence of some degree of root resorption without orthodontic treatment and more than 90 per cent of teeth had radiographic evidence of root resorption following orthodontic treatment.

Levander and Malmgren[45] examined 390 maxillary incisors and found one per cent had greater than one third of root resorption and concluded that significant root resorption following orthodontics was a rare event. However, Mirabella and Artun[46] studied a sample of 343 consecutively treated adults with standardised periapical radiographs and found that 40 per cent of the patients had one or more teeth with 2.5 mm resorption or greater. Lupi *et al*[47] investigated a sample of 88 ethnically and racially diverse adults radiographically and revealed that 15 per cent of the teeth had resorption prior to orthodontic treatment. After 12 months of orthodontic treatment, the incidence of OIIRR increased to 73 per cent. Two per cent showed moderate to severe root resorption before treatment and 24.5 per cent showed the same degree of severity after orthodontic treatment. Killiany[48] reported external apical root resorption (EARR) of greater than 3 mm to occur at a frequency of 30 per cent with only five per cent of treated individuals found to have greater than 5 mm of root resorption. Later, in a large study of more than 868 cases, greater than 2 mm of EARR was seen in 25 per cent of treatment patients.[49] The most recent study by Smale *et al*,[50] showed 24 per cent of the teeth expressed root shortening, but only 3.6 per cent had shortening of more than 2 mm.

4.3 Location of Orthodontically Induced Inflammatory Root Resorption

Stenvik and Mjor[38] found 60 per cent of teeth in experimental material had evidence of resorption of cementum and dentine and the distribution in the marginal and apical parts of the tooth was the same.

Generally, the distribution of root resorption is dictated by the pressure zone created by different types of tooth movement. However, OIIRR tends to occur preferentially in the apical region because:

- the force concentrates at the root apex due to orthodontic tooth movement being not entirely translatory and the fulcrum is usually occlusal to the apical half of the root;[51]
- the orientation of the periodontal fibres in the apical end is different which increases the stress in the region;[43]
- more friable acellular cementum covers the apical third of the root which can be easily injured in the case of trauma and concomitant vascular stasis;[43,51,52]

EARR is a special term that describes OIIRR at the apical region.

4.4 Severity of Orthodontically Induced Inflammatory Root Resorption

Brezniak and Wasserstein[31] have illustrated three levels of OIIRR severity:

1. Cemental or surface resorption with remodelling.

   This type of OIIRR is similar to trabecular bone remodelling in which the resorbed cemental layers are fully regenerated or remodelled.

2. Dentinal resorption with repair.

   The resorbed cementum and the outer layers of the dentine are repaired with cementum material which may or may not restore the root to its original size and shape.

3. Circumferential apical root resorption.

Significant resorption of the root apex results in root shortening with no evidence of regeneration. However, with time the sharp edges formed by resorption craters may be levelled. Surface reparation only occurs in the cemental layer.

4.5 Diagnosis of Orthodontically Induced Inflammatory Root Resorption

Radiographs have been widely used to clinically diagnose OIIRR. Dental panoramic tomograph (DPT) provides an overall view of the dentition with lower radiation dose than a full-mouth series of intraoral radiographs.[53] However, due to the narrowness of the focal trough, the apices and palatal structures can be out of focus in the incisor region. Therefore, additional radiographs such as periapical radiographs or occlusographs may be needed to supplement the DPT. Periapical radiographs are more readily used in research studies which investigate the extent of OIIRR based on the premise that these radiographs provide greater detail and less distortion. Sameshima and Asganfar[54] compared periapical radiographs with DPT and found the amount of root resorption was exaggerated by 20 per cent or more on a DPT. Paralleling technique for periapical radiograph is preferable as it provides a geometrically accurate image and together with the use of a film holder and aiming device, radiographs can be standardised in two different time points. Lateral cephalograms have also been used to diagnose root resorption but the image overlap renders its accuracy in quantitative evaluation. The 2D nature of radiography limits its accuracy because buccal and lingual root defects are not detectable.[55] Computed tomographic scanning offers a significant advantage over conventional radiography in the detection and quantification of root resorption. However, its high cost and high radiation exposure to the patient limit its use to mainly *in vitro* research. With the advance of technology, cone beam computed tomography will be more readily used in future root resorption studies. It will provide a more accurate 3D image with a lower dosage of radiation which can be used for qualitative and quantitative assessment. Heimisdottir *et al*[56] have evaluated the accuracy in using plain

radiographs in detection of root resorption. The results of the study suggested the use of computer tomography in root resorption investigation, as the severity of root resorption cannot be accurately judged from plain radiographs alone.[56]

4.5.1 Qualitative Radiographic Diagnosis

Qualitative assessment of OIIRR can be achieved from periapical radiographs. The severity of the OIIRR is based on a subjective scoring system. In an earlier study by Massler and Malone,[44] their assessment system was based on Hemley[57] which has ten different types of resorption (Appendix 1). Malmgren *et al*[58] has devised a more simplified subjective scoring system which has been used by several studies (Appendix 2).[45,59,60] The radiographic appearance of OIIRR is characterised by rough, jagged and notched contours of the root especially at the apical region.[60] The concurrent widening of periodontal space accentuates the rough appearance. [60]

4.5.2 Quantitative Radiographic Diagnosis

Periapical radiographs can also be used in quantitative analysis of OIIRR. The image distortion between pre- and post- treatment radiographs are corrected using crown length registrations.[61-64] The length of the root from CEJ to apex is measured on pre and post-treatment periapical radiographs. Any change in the root length is corrected for radiographic magnification differences with a ratio between crown lengths in the before and after films. This method is reasonably accurate but has some weaknesses. It relies on locating the CEJ, which can be susceptible to interpretation by the observer. Furthermore, because the CEJ changes height moving circumferentially around the tooth, variation in x-ray beam angulation with respect to the tooth will project the CEJ differently.

4.6 Pathogenesis of Orthodontically Induced Inflammatory Root Resorption

OIIRR is associated with local overcompression of the PDL which results in an aseptic coagulation necrosis process i.e. hyalinisation. This was confirmed by a 2D finite element model (FEM) study that showed the pattern of root resorption was related to compression in the PDL.[65] Resorption of the cementum occurs simultaneously with the removal of hyalinised tissue.[6,66-68] Resorption starts at the periphery of the hyalinised periodontal membrane which is followed by resorption of surrounding root and bone surfaces[4,69] and invasion of blood vessels.[12] The resorption process propagates until no hyalinised tissue is present and or the force level diminishes. The resorption crater expands the root surfaces involved and thereby indirectly decreases the pressure exerted through force application. Decompression alters the process to reverse and cementum reparative process begins. Rygh[6] discovered the elimination of hyalinised tissue in the human PDL completed after 20 to 25 days but root resorption increased after this time. It has also been documented that ERR initiated 10 to 20 days after force onset[38,39,67] and continued even during extended retention periods of up to one year.[8,9,70] The resorbing areas on a root surface may show signs of concurrent active resorption and repair.[8,38]

4.6.1 Protective Role of Cementum and Periodontal Tissues in the Pathogenesis of Orthodontically Induced Inflammatory Root Resorption

The surface of the cementum is more resistant to resorption than dentin. This resorption resistant property is attributed to the poor ability of the odontoclasts to

remove the more mature collagen nature and unmineralised precementum layer of the surface cementum.[6,68] It has also been suggested that the root resorption process was related to signals to, or damage of the cells and other barriers that protect the root surface. Lindskog and Hammarstrom[71] speculated that the root was protected against resorption by a potent collagenase inhibitor in the normal PDL and cementum. The synthesis of the collagenase inhibitor discontinued when the PDL and cementum were severely damaged which resulted in the inability to control the collagenase activity in the microenvironment.

The presence and vitality of periodontal fibres seem to protect the cementum and perhaps more importantly the epithelial cell rests of Malassez (ECRM). These rests are remnants of HERS and are found close to the cementum.[17] As reviewed by Löe and Waerhaug,[72] the presence of these epithelial rests played a role in the maintenance of the periodontal membrane and exerted a protective function against replacement root resorption.

In a classic study of cementum, Kronfeld[73] noted that the cementum protected the integrity of the root and appeared thinner in areas of greater functional stress. With teeth already exhibiting root blunting at the start of treatment, the apex is only partially covered with cementum and may even be cementum free. These teeth may be at greater risk of resorption during orthodontics than intact cases.

Vandevska-Radunovic *et al*[74] investigated the effect of experimental tooth movement on nerve fibres. Immunohistochemistry revealed that nerve fibres were not found in tissues near cellular cementum and root resorption craters. This occurred at the beginning of root resorption (seven and 14 days). However, by 21 days, nerve

fibres were found entering and within root resorption craters which were coincident with the regenerative activity of the periodontal tissues. The authors speculated that under physiological conditions, the nerve fibres might balance the resorptive and reparative activities of the hard tissue forming cells and provide a protective role on the root surface.

4.6.2 Light Microscopy of Orthodontically Induced Inflammatory Root Resorption

Due to ethical and practical issues, limited amount of histological studies have investigated the cellular activities related to OIIRR in humans. Therefore, the majority of the studies were carried out in rodents which allowed investigation not only on the surface of an extracted tooth, which was only possible in humans, but also the surrounding alveolar bone and periodontal membrane. Brudvik and Rygh[4,12] have performed extensive rodent research on the histological sequence of root resorption and repair under light microscopy and TEM. These studies have shown that cellular activities within the periodontal apparatus varied with time and location. The process of OIIRR was described as three sequences of events in two different locations, periphery and main hyalinised zones:

1. Tartrate resistant acid phosphatase (TRAP) negative mono-nucleated fibroblast-like cells initiated root resorption from the periphery of the main hyalinised zone. This early resorptive phase seemed to be initiated by the nearest viable cells in the presence of adequate vascularity.
2. TRAP positive multi-nucleated cells participated in the removal of main hyalinised tissue and resorption of the adjacent root structure. This later phase commenced only after a considerable amount of hyalinised tissue

between alveolar bone and the root surface had been eliminated and continued even after the initial root resorption had terminated. The cells involved during this resorptive phase were derived from adjacent marrow spaces.

3. Active root resorption continued to occur in areas of hyalinised tissue even after orthodontic force had ceased. Reparative process occurred from the periphery of the resorption craters and extended to the central part.

Appendices 3 and 4 describe, in detail, the sequence of cellular events in the periphery zone and main hyalinised zone respectively.[4,12]

4.6.3 Scanning Electron Microscopy of Orthodontically Induced Inflammatory Root Resorption

Enhanced visual and perspective assessment of OIIRR can be achieved by SEM. SEM also allows 2D measurement of resorption craters using surface area landmarks. Chan *et al*[75] compared 2D versus 3D measurements of the root resorption craters over 28 days and demonstrated no significant difference even though measurements were made on the curved root surfaces.

Kvam[67] was one of the earlier investigators who utilised SEM to depict and measure root resorption craters. Marginal root resorption began at 10 days and root resorption craters initiated as small cavities with diameters of 6 μm. As resorption progresses, the number of small, round and thin walled craters increased and merged into extensive and shallow craters. Initially, the cervical portion of the root was most frequently affected but subsequently resorption craters were found at the middle

root portion. Latticed fibres appeared on the resorption surfaces of the active resorption sites indicating that the inorganic minerals were removed prior to the collagen component of dentine and cementum. All teeth that had been moved for a period longer than 15-20 days showed marginal root resorption and in cases observed longer than 25 days the resorption extended into dentine. Root resorption occurred beneath the hyalinised zone and the craters were found to be most extensive in this area.

Harry and Sims[39] studied the topography of root resorption on human bicuspids that were subjected to intrusive forces using SEM. In 14 days, small apical resorption sites were detected and some had already coalesced to form larger resorption craters. Within 35 days of the experiment, shortened root lengths were observed. Progressive apical resorption accompanied by regions of cellular cementum repair was found after 70 days. The edges of resorption craters were repeatedly observed to pass through rather than around the Sharpey fibres in contrast to an earlier study by Jones and Boyde.[76]

Barber and Sims[8] examined the topography of root resorption craters when the human teeth were moved by rapid maxillary expansion (RME) appliances. The experimental teeth that were activated and retained for a short time had small areas of active resorption and repair confined mainly to the cervical regions of their buccal root surfaces. Experimental teeth that were activated and retained for 20, 33 or 36 weeks showed large resorption craters scattered along the entire buccal surface. Actively resorbing surfaces were smooth and multilocular in appearance and were delineated by a rim of relatively sheer and undermined cementum. Resorbing dentine was easily distinguished from resorbing cementum due to the presence of

minute surface openings demarcating sites previously occupied by odontoblastic processes.

Recently, Mavragani *et al*[77] investigated early root alterations after orthodontic force application on rats. They described three morphological types of resorption craters: small, shallow and deep. Small isolated craters were often seen scattered on the controlled teeth which had not been subjected to any force. The undamaged cementum displayed mosaic-like surface pattern and mineralised projections. In the experimental teeth, small craters were often observed with shallow resorption bays. As the experiment progressed, the size of the resorption crater decreased from 6.7μm diameter to 5.0μm in four days. The cementum near the shallow type of craters had a smooth appearance without mineralised projection. The deep resorption craters were found in areas of existing shallow resorption craters. This type of resorption occurred as an undermining process leaving sharp edges at the periphery. Vascular elements were also observed near these craters.

4.6.4 Transmission Electron Microscopy of Orthodontically Induced Inflammatory Root Resorption

Brudvik and Rygh[2,69] further studied ultrastructurally the cellular events of OIIRR in a rodent model. From six hours to two days after orthodontic tooth movement, macrophage-like cells characterised by indented nuclei, a high number of mitochondria, few pseudopodia and scarce endoplasmic reticulum (ER) appeared in the middle of the PDL. Loss of surface of cementum was evident and fibroblast-like cells were observed close to the fibre fringe or in contact with the subjacent mineralised cementum. During three to five days, mononucleated

fibroblast-like cells with large nuclear volume, little or no intracellular collagen material and a high number of densely stained bodies were regularly observed on the root surface. Larger cells with a diameter of 20 to 50 μm, mono- or bi-nucleated, were observed in close contact with the mineralised cementum. These cells revealed numerous mitochondria and internal cisternae as well as varying surface characteristics when in contact with mineralised cementum. The majority of these cells showed no ruffled border on the mineralised cementum surface but were rich in internal clear vacuoles. Occasionally, multi-nucleated cells with ruffled border resorbed mineralised cementum and dentine. Disintegration of vascular, cellular and fibrillar elements occurs during the first seven days.[78-80] From seven to 14 days, multinucleated TRAP positive cells were still near or in contact with the root surface beneath partly eliminated necrotic tissue. These cells showed many mitochondria and vacuoles including surfaces characterised by a varying number of cup-like cytoplasmic processes. No ruffled border was observed when these cells were away from any mineralised surface. From 14 to 21 days, a thin layer of cementum like material was observed in the root resorbed craters. Sharpey's fibres inserted into the newly formed cementum layer and mono-nucleated fibroblast like cells were seen in the fibre-rich PDL.

Animal models presented several differences in the anatomical and histological pattern when compared to humans.[81,82] Faltin *et al*[13] performed an ultrastructural study using TEM to analyse the human cementum and PDL when subjected to intrusive force. Their findings were in agreement with previous study by Rygh[6] which was characterised by:

- Different stages of degeneration of cells (fibroblasts, cementoblasts and cementocytes) as displayed by unstructured nuclear chromatin with pyknotic

nucleus, electron opaque and partial segmentation;

- Increased electron opacity in the cytoplasm which was evidenced by numerous microfilaments, disarranged and limited organelles with excessive dilation of the rough endoplasmic reticulum (rER) cisterns;
- Loss of the plasma membrane and cell fragmentation;
- Loss of the fibrillar nature of PDL and a granular and amorphous appearance of the interfibrillar components;
- Loss of cementoid due to degeneration of cementoblasts and non-mineralised extracellular matrix;
- Irregular contour and loss of the limiting lamina in the boundaries of the cementocyte lacunae near the PDL;
- Reduction of the space between the cementocyte cell body and the mineralised surfaces of the lacunae;
- Mono- and multi-nucleated macrophage-like cells in hyalinised PDL near the cementum. These cells contained several phagosomes, lysosomes, digestive vacuoles and a large number of well-developed rER which were responsible for the removal of necrotic tissue;
- Clast-like cells with cytoplasm containing lysosomes and vacuoles, and clear zones, without ruffled borders. They were normally near the hyalinised zone, participated in the process of removing necrotic elements and also responsible for cementum resorption;
- Narrowed blood vessels with altered lumen shape. Bulky endothelial cells with endothelium discontinuity and extravasation of blood cells. This resulted in haemorrhage and oedema in the PDL near the hyalinised zone;
- Evidence of angiogenesis and revascularisation of the PDL which was a sign of vascular repair.

4.6.5 Biochemistry and Molecular Biology Related to Orthodontically Induced Inflammatory Root Resorption

Resorption activity in response to mechanical or chemical stimuli by the cells of the PDL, is characterised by the synthesis of prostaglandin E2 (PGE_2) with a concomitant increase in cyclic adenosine monophosphate.[83] This process is regulated by hormones (parathyroid[83,84] and calcitonin[84,85]), neurotransmitters (substance P,[86] vasoactive intestinal peptide[87] and calcitonin-gene related peptide[88]) and cytokines or monokines (interleukin-1-alpha,[84,88] interleukin-1 beta (IL-1β),[89,90] interleukin-2,[91] tumour necrosis factor (TNF),[84,91] and interferon-gamma).[92] It has also been shown that the osteoclasts are controlled by osteoblasts in many ways.

Recent studies have shown the role of osteoprotegerin (OPG) and receptor activator of nuclear factor kappa B ligand (RANKL) in osteoclastogenesis[93] and hence the possibility of their role in root resorption.[94] An increase in RANKL and a decrease in the level of OPG expression in physiologic root resorption in deciduous human teeth were found. It was then postulated that the expression of RANKL regulated by the decoy receptor OPG had direct involvement in osteoclastogenesis and therefore the activation of physiologic root resorption. Low *et al*[95] investigated the role of OPG and RANKL in artificially induced root resorption in a rat model. Tooth movement was induced by heavy forces and reported an increased level of both RANKL and OPG at sites adjacent to resorption zones compared to the control teeth with no resorption. Recently, Yamaguchi *et al*[96] investigated the effect of compressive force on the production of RANKL and OPG. The findings revealed an increase of RANKL and a decrease of OPG in the severe root resorption group. Kojima *et al*[97] have shown that Substance P stimulated the production of PGE_2 and

RANKL which may be responsible for OIIRR.

4.6.6 Physical Properties of Orthodontically Induced Inflammatory Root Resorbed Cementum

Cementum at the cervical and middle thirds of the root has greater hardness and elastic modulus than that of the apical third.[98,99] This is because of the variable mineral content of cellular and acellular cementum. It was also found that hardness was positively correlated to the amount of mineralisation. Chutimanutskul[100] conducted a study that assessed the relationship between the magnitude of orthodontic forces and physical properties of the human cementum. The study revealed the mean hardness and elastic modulus of cementum was greater in the light force group than the heavy force group. The mean hardness and elastic modulus of cementum gradually decreased from the cervical to apical regions as observed in previous studies.[98,99] This study concluded that the hardness and elastic modulus of cementum were affected by the application of orthodontic forces.

Rex *et al*[101] studied the mineral composition (Calcium (Ca), Phosphorus (P) and Fluoride(F)) of human premolar cementum following the application of orthodontic forces. The results showed limited change in the mineral composition of cementum after the application of light force. There was a trend towards an increase in the Ca and P concentration of cementum at various areas of PDL compression. The application of heavy force caused a significant decrease in the Ca concentration of cementum at certain areas of PDL tension. Orthodontic force did not appear to influence the F concentrations in cementum.

4.7 Factors Affecting Orthodontically Induced Inflammatory Root Resorption

OIIRR can be influenced by a wide range of shared biological and or mechanical factors. Biological factors include those that are not within the control of the clinician and are directly related to the patient. Some of these factors may be within the control of the patient which indicated that they can be environmental or genetic in origin. Mechanical factors are attributed to the nature of the orthodontic appliance and could be controlled by both the clinician and the patient.

4.7.1 Biologic Factors

4.7.1.1 Genetic Factors

Root resorption can occur in normal individuals without orthodontic treatment which illustrates the individual susceptibility to root resorption.[43] In fact, root resorption is related to one's tissue response and metabolic activity. The metabolic signals (e.g. hormones, body type and metabolic rate) influence the relationship between osteoblastic and osteoclastic activity which modifies cell metabolism, an individual's reaction pattern to disease, trauma and ageing.[6] Root resorption can vary among individuals and within the same person at different times.

Genetic influence on the susceptibility to root resorption remains controversial. Newman[102] reported family clustering of EARR but the pattern of inheritance was not clear. Harris *et al*[103] investigated the genetic influence on EARR by using sib-pair model and reported 70 per cent heritability for resorption of maxillary incisor roots and the mesial and distal roots of the mandibular first molars. This

accounted for approximately half of the total phenotypic variation.[103] This meant that siblings experienced similar levels of EARR in response to orthodontic treatment. A recent study has also revealed a familial association of OIIRR.[104]

Al-Qawasmi *et al*[105] identified a key role of IL-1β gene polymorphism for a genetic influence in EARR in orthodontically treated individuals. Individuals homozygous for the IL-1β (+3953) allele 1 had a 5.6 fold increased risk of EARR >2mm as compared with individuals who are not homozygous for the IL-1β (+3953) allele 1. This polymorphism accounted for approximately 15 per cent of variation in EARR of upper centrals. Harris *et al*[103] and Hartsfield *et al*[106] indicated that since approximately half of the variation in EARR was influenced by genetic factors, and that variation at IL-1β accounts for only 15 per cent of phenotypic variation, there must be other genes that influenced EARR. Another candidate gene for EARR is TNFRSF11A, which encodes the receptor activator nuclear factor-kappa B, an essential signalling molecule in osteoclast formation and activation.[107]

Rossi *et al*[108] examined IL-1β and TNF-α production in monocytes from a group of orthodontic patients with severe root shortening and found no significant differences in mean levels between resorption and non-resorption groups. This supported the likelihood that EARR was genetically heterogeneous.

One of the difficulties in assessing the genetic contribution to OIIRR is the ability to separate genetic factors from environmental factors such as orthodontic treatment.[109] Ngan *et al*[109] investigated the genetic contribution to OIIRR by retrospectively assessing the pre- and post-treatment records of 16 monozygotic and 10 dizygotic twins. Each twin pair had the same malocclusion and the same type of appliance

treatment with the same clinician. Panoramic radiograph tooth root measurements were used to obtain quantitative and qualitative estimates of concordance for EARR. The concordance estimate for root resorption in monozygotic twins was 44.9 per cent for qualitative measurements and 49.2 per cent for quantitative measurements. The concordance estimate for root resorption in dizygotic twins was 24.7 per cent for qualitative measurements and 28.3 per cent for quantitative measurements. The authors thus confirmed the presence of a genetic component to OIIRR. However, they advised that a larger sample was required before a model of heritability could be used to determine the components contributing to the variance.[109]

Ethnicity can also influence the susceptibility to OIIRR. Sameshima and Sinclair[49] have found that Caucasians and Hispanics were more prone to OIIRR than Asians.

4.7.1.2 Environmental Factors

4.7.1.2.1 Systemic Factors

Davidovitch *et al*[110] hypothesised that individuals who had medical conditions that affected immune system may be at high risk for developing excessive root resorption during the course of orthodontic treatment.

4.7.1.2.1.1 Asthma and Allergy

There was an increased incidence of OIIRR, especially blunting of maxillary molars, found in patients with chronic asthma.[111,112] This could be attributed to the close proximity of the roots to the inflamed maxillary sinus and or the presence of inflammatory mediators in these patients. McNab *et al*[111] reported an elevated incidence of EARR in the asthma group but both asthmatics and healthy patients

exhibited similar amounts of moderate and severe root resorption. This implied that the increased incidence of EARR in asthmatics was confined to an increase in root blunting. Therefore, asthmatics had only a minimal risk to posterior EARR that may not adversely affect the function or longevity of the posterior teeth.

Allergy may increase the risk of OIIRR.[112,113] Owman-Moll and Kurol[114] analysed the factors that might be associated with OIIRR and found subjects with allergy showed an increased risk of root resorption but this was not statistically significant. Nishioka *et al*[115] investigated the association between excessive root resorption and immune system factors in a sample of Japanese orthodontic patients. The result showed that the incidence of allergy, asthma and root morphology abnormality was significantly higher in the root resorption group.

Corticosteroids are commonly used to treat allergy, asthma, dermatitis and eczema. It has been shown to interfere with orthodontic tooth movement rate and tissue reaction in animal studies.[116-119] Research on the effect of orthodontic treatment under corticosteroid treatment on root resorption remains controversial. This could be due to different dosages of corticosteroids used and also different animal models studied. Ashcraft *et al*[118] injected osteoporotic dosage of cortisone acetate (15 mg/kg) in young rabbits and revealed significantly more rapid orthodontic tooth movement and greater tendency to root resorption in the pressure areas. Later, Ong *et al*[119] prescribed short term treatment with 1 mg/kg oral prednisolone in rats and showed significantly less root resorption. This suggested a suppressing role of the drug on clastic activities. Recently, Verna *et al*[120] investigated the effect of acute and chronic corticosteroid treatment on OIIRR. The results showed more root resorption in the acute treatment group on the mesial coronal level than chronic

treatment and control groups. The less resorption found in the chronic group may be due to faster remodelling of bone, less hyalinisation and less remodelling of root tissue.

4.7.1.2.1.2 Endocrine and Hormone Imbalance

The endocrine system is closely related to bone activity and metabolism. Imbalance of the endocrine system due to hypothyroidism, hypopituitarism, hyperpituitarism, hyperparathyroidism,[121] Paget's disease[122] and hypophospataemia[123] are hypothesised to be related to OIIRR.[121,124,125] Goldie and King[126] reported an association with a decrease in OIIRR and secondary hyperparathyroidism, while the opposite was found by Engstrom *et al.*[121] An excess of thyroid hormones which increased bone turnover have been found to reduce root resorption during orthodontic tooth displacement in a rat model.[127] However, hypothyroidism has been associated with increased root resorption in the absence of orthodontic load.[128] Smith[122] published a case report of a patient who had Monostotic Paget's disease of the mandible with progressive resorption of teeth over a period of seven years. Hypercementosis of the tooth root is common in patients with Paget's disease. However, Smith[122] suggested that root resorption may accompany or precede hypercementosis.

Calcitonin can inhibit odontoclast activity.[129] The action of calcitonin on osteoclast occurs at later stages of osteoclast development and it inhibits the fusion of pre-osteoclasts to form mature multinucleated cells.

4.7.1.2.1.3 Alcohol Consumption

The presence of ethanol in the circulation inhibits the hydroxylation of vitamin D3 in the liver, thus hindering calcium homeostasis and resulting in a rise in parathyroid hormone (PTH). PTH in turn enhances the resorption of mineralized tissues including tooth roots. It has thus been suggested that chronic alcohol consumption during orthodontic treatment increased the risk of severe OIIRR.[113]

4.7.1.2.1.4 Nutrition

Root resorption has been demonstrated in animals deprived of dietary calcium and vitamin D.[121,125] Engstrom *et al*[121] found the experimental rat group that was fed with a diet deficient in calcium and vitamin D had hypocalcaemia, increased alkaline phosphatase activity and increased circulating PTH when compared to the control group which was fed with a normal diet. Orthodontic tooth movement produced greater and more rapid bone resorption and more severe OIIRR in the experimental group. The study also showed an increased number of osteoclasts in the PDL of the test group which suggested to be due to the increased PTH levels.

It has been proposed by other authors that poor nutrition was not a major aetiological factor in OIIRR.[126] Goldie and King[126] deprived lactating rats of dietary calcium and phosphorus and compared them with a control group on a normal laboratory diet. Despite a greater amount of tooth movement and loss of bone mass in the dietary deficient group, the OIIRR craters were smaller.

4.7.1.2.1.5 Drugs

Inhibiting cyclo-oxygenase and the subsequent production of prostaglandins with non-steroidal anti-inflammatory drugs (NSAIDs) can be useful in decreasing bone and root resorption. Villa *et al*[130] investigated the effect of Nabumetone, a type of NSAIDs, on root resorption during intrusive orthodontic tooth movement. The results showed less root resorption when patients received Nabumetone and the drug did not impede tooth movement. This was supported by Kameyama *et al*[131] in which the rats were prescribed aspirin and led to a suppression of root resorption caused by mechanical injury.

Bisphosphonates are potent inhibitors of bone resorption which are widely used to treat osteoporosis. Bisphosphonates directly or indirectly induce apoptosis in osteoclasts which played a role in the inhibition of bone resorption.[132] Liu *et al*[133] investigated the effect of Clodronate, a non-nitrogen containing bisphosphonate, on orthodontic tooth movement and root resorption in rats and found tooth movement was less, number of osteoclasts decreased and root resorptive area was smaller. Clodronate may have either inhibited recruitment of osteoclast or promoted osteoclast apoptosis.[134] Clodronate has also been shown to inhibit the production or release of pro-inflammatory molecules in macrophages and or osteoblastic cells.[135-139]

4.7.1.2.1.6 Ehlers-Danlos Syndrome

Ehlers-Danlos syndrome is a group of generalised disorders that is characterised by abnormalities of the connective tissue leading to fragility of the skin and blood vessels, hyper-extensibility of the skin and joint hypomobility[140] Patients suffered from Ehlers-Danlos syndrome were found to be at high risk of OIIRR.[141]

4.7.1.2.1.7 Psychologic Stress

Davidovitch *et al*[110] recorded a radiographic study of OIIRR patients and their pair-matched controls. The results revealed that patients who experienced psychological stress had a higher incidence of excessive root resorption.

4.7.1.2.1.8 Chronological Age

The ageing process results in changes to the hard and soft tissues of the dentoalveolus. The periodontal membrane becomes less vascular, aplastic and narrow with age. The bone becomes more dense, avascular and aplastic and the thickness of cementum increases.[7] Reitan[7] proposed that these changes were responsible for the increased incidence of root resorption found in adults. Adults are also exposed to previous orthodontic treatment and inflammation associated with endodontic treated teeth or traumatised teeth which predisposes them to OIIRR.[61,142]

It is a traditional belief that OIIRR increases with age,[68,143] however, some studies contradict this thought.[52,63,144,145] Mirabella and Artun[46] evaluated a large sample of adult patients for their prevalence and severity of OIIRR in maxillary anterior teeth. Forty per cent of the adults had one or more teeth with ≥2.5 mm resorption. This indicated that the sample of adults had a higher mean value of severely resorbed tooth per patient than the comparable group of adolescents which was 16.5 per cent.[61] Lupi *et al*[47] found the number of incisors showing root resorption was 73 per cent after orthodontic treatment and 24.5 per cent had moderate to severe apical root resorption. On the other hand, Harris and Baker[144] reported that 61 per cent of the adult patients had some degree of resorption after orthodontic treatment which was not significantly different from the 58 per cent of adolescent patients experiencing root resorption in that same study.

Linge and Linge[61] found less resorption in patients treated before the age of 11. They suggested that resorption could be avoided if tooth movement was completed before the roots were fully developed, before the age of 11.5 years, but treatment at this age was impossible for many patients.

4.7.1.2.1.8 Dental Age

Partially formed roots have been found to develop normally during orthodontic treatment and it has been suggested that teeth with open apices may be more resistant to EARR.[41,61,143,146] Mavrangani *et al*[147] investigated root lengthening during orthodontic treatment in relation to the age of patient, the developmental stage of the root and the anticipated growth. Results revealed a definite advantage for younger teeth with regards to post-treatment root length. In contrast to these findings, Hendrix *et al*[148] discovered that the teeth with incomplete root formation at the onset of orthodontic treatment did not reach normal tooth length. The design of this study has been criticized by others as it relied on panoramic radiographs with no adjustment for radiographic projection differences between pre- and post-treatment films and thus the results could be questionable.[149]

4.7.1.2.1.9 Gender

Some studies have suggested that orthodontically treated females had a greater incidence of OIIRR than males.[102,150-152] Newman[102] found a ratio of OIIRR susceptibility of 3.7 to 1 for females to males respectively. Dougherty[153] suggested that this finding might be due to the differences in root maturity between males and females at the age of tooth movement. Few studies have shown the opposite that males have higher incidence of OIIRR than females.[52,154] However, most of the studies have found no correlation between gender and the extent of

OIIRR.[49,51,63,103,145,155,156]

4.7.1.2.2 Local Factors

4.7.1.2.2.1 Habits

A number of habits have been reported to result in an increased risk of OIIRR. Finger sucking beyond the age of seven years has been suggested to be a risk factor.[61] Nail-biting,[157] forward tongue pressure and tongue thrust[61,102] were also proposed to be linked. Some studies[44,158] found that the root resorption was significantly higher before orthodontic treatment in nail biting patients whereas others[143] found no greater incidence of root resorption in nail biting patients. Long-term orthopaedic tongue thrusting forces which resulted in anterior open bite may promote root resorption,[159] particularly when vertical elastics were used in an attempt to close open bites associated with tongue problems.

4.7.1.2.2.2 History of Trauma

Orthodontic tooth movement of a severely traumatised tooth may result in increased resorption.[58,61,143,160,161] Linge and Linge[143] found that teeth which had previous trauma had an average loss of root structure after orthodontic movement of 1.07 mm compared with a loss of 0.64 mm for untraumatised teeth. However, Kjaer[151] proposed that teeth with slight or moderate injuries may not have any greater tendency towards OIIRR than uninjured teeth. Malmgren[58] suggested a waiting period of one year after a traumatic incident before the initiation of orthodontic tooth movement.

4.7.1.2.2.3 Density and Turnover of Alveolar Bone

It has been suggested that OIIRR was amplified in dense alveolar bone compared to less dense alveolar bone, especially if there was an increased number of resorptive cells associated with the increased number of marrow spaces.[7,68] Reitan[68] proposed that a strong continuous force on low density alveolar bone caused an equivalent amount of OIIRR to that of a mild continuous force on high density alveolar bone.

Kaley and Phillips[162] identified the risk of root resorption was 20 times greater when upper incisors were in close proximity to cortical plate. Horiuchi *et al*[152] also found that EARR of maxillary central incisor was influenced by root approximation to the palatal cortical plate during orthodontic treatment. The study also suggested root approximation to the palatal cortical plate during orthodontic treatment could explain approximately 12 per cent of the variance observed in the level of root resorption and the maxillary alveolar bone width could explain approximately two per cent of the variance.

On the contrary, Otis *et al*[163] found that the amount of alveolar bone present around the root, the thickness of cortical bone, the density of trabecular network and fractal measurements on the bony trabeculae had no significant correlation with the amount of root resorption.

Verna *et al*[164] investigated the impact of bone turnover rate on the amount of tooth movement and the incidence of OIIRR in rats. High bone turnover increased the amount of tooth movement compared with the normal or low bone turnover state. The untreated side in the low bone turnover group showed more root resorption suggesting that in clinical situations where turnover of alveolar bone was delayed,

root surfaces could already be affected by root resorption at baseline condition.

4.7.1.2.2.4 Types of Malocclusion

A number of studies have found a relationship between OIIRR and malocclusion.[49,61,103,159] Severe malocclusion requires greater tooth movement, for example, greater overjet requires greater retraction and deeper overbite needs greater intrusion.[103,145] Hence, greater amount of root resorption.

Taner *et al*[165] examined the differences in the amount of root resorption following extraction therapy in subjects with Class I and Class II Division 1 malocclusions. The results showed a mean of approximately 1 mm of apical root shortening in Class I patients and the mean root resorption was more than 2 mm in Class II division I subjects. In contrast, VonderAhe[166] found approximately the same amount of root resorption in both Class I and Class II subjects.

Kaley and Phillips[162] reported that Class III patients showed severe root resorption with root apex approximation to the palatal cortical plate. They suggested that the maxillary incisors were tipped forward in compensation for the Class III jaw relationship which forced the roots against the palatal cortical plate during orthodontic treatment. Class III surgical cases demonstrated 1.6 per cent and 20.8 per cent reduction in maxillary and mandibular incisor lengths respectively.[162] It has been proposed that orthognathic surgery contributed to OIIRR by altering the blood supply and nutrition to the periodontium.[167]

Harris and Butler[159] demonstrated that open bite patients possessed significantly greater degrees of root resorption. It was believed that the orthopaedic forces of

tongue thrusting generated the same physiologic responses as mechanotherapy intended to torque or intrude a tooth. This study also showed that the severity of the malocclusion was a valuable predictor:

1. The greater the overjet, the greater the in-treatment root loss
2. The great the skeletal discrepancy, the greater the resorption
3. The more the palatal plane was tipped up anteriorly, the greater the resorption
4. The steeper the Down's occlusal plane, the greater the observed degrees of incisor root shortening.

4.7.1.2.2.5 Hypofunctional Periodontium

Hypofunctional periodontium results in narrowed periodontal space and derangement of functional fibres which would eliminate the normal cushioning effect of the PDL[168] thus resulting in a high concentration of force. This leads to stimulation of inflammation by the promotion of inflammatory mediators secreted from local cells to induce destruction of tooth and bone.[169]

Sringkarnboriboon *et al*[170] compared the amount of root resorption associated with a normal and a hypofunctional periodontium in rats during experimental tooth movement cause by heavy continuous force. The results showed the amount of root resorption was significantly greater in teeth with a hypofunctional periodontium than in those with a normal periodontium. Similarly, Terespolsky *et al*[171] discovered a delay in recovery of PDL in hypofunctional teeth. The distortion of the PDL in the hypofunctional teeth remained comparable with that caused by spring activations. Therefore, physiologic function is imperative for rapid recovery of the distorted PDL and healing of dental and periodontal lesions. These results suggested that orthodontic movement of non-occluding teeth should be performed with caution.

4.7.1.2.2.6 Occlusal Trauma

Occlusal trauma from improper occlusion, interfering dental restorations and inadequate prosthetic appliances accelerated root resorption.[172,173] Functional trauma to individual teeth caused root resorption, however, 85 per cent of these areas showed anatomically complete repair with secondary cementum.[174]

4.7.1.2.2.7 Missing Teeth

One group of authors has found that patients with multiple tooth aplasia (four or more missing teeth) were more prone to OIIRR especially teeth with irregular root morphology and protracted treatment with elastics and rectangular arch wires.[59]

4.7.1.2.2.8 Specific Tooth Vulnerability to Root Resorption

The teeth most frequently affected by OIIRR according to severity are the maxillary lateral incisors, maxillary central incisors, mandibular incisors, distal root of mandibular first molar, mandibular second premolars and maxillary second premolars.[49,175-177] Maxillary lateral incisors are more susceptible to root resorption due to the abnormal root shape.[49] Mirabella and Artun[178] revealed upper central incisors with anomalous roots had increased vulnerability to OIIRR. Maxillary teeth showed a higher incidence of root resorption than mandibular teeth.[34,60,102,155,179]

4.7.1.2.2.9 Dental Invagination

Dental invagination is the most prevalent dental anomaly in orthodontic patients.[180] Maxillary lateral incisors are most often affected followed by maxillary central incisors. It has been claimed that dental invagination was one of the predisposing factors for OIIRR.[151,180] However, there is no general agreement concerning the

role of dental invagination as a risk factor for orthodontic root resorption.

Kjaer[151] examined the pre-treatment radiographs of 107 patients selected by orthodontic practitioners for having experienced severe apical root resorption. The frequencies of tooth agenesis, peg-shaped or small maxillary lateral incisor crown, crown invaginations, ectopic eruption and taurodontism ranged from three to 40 per cent in the sample. Kjaer,[151] therefore, suggested that dental anomalies may be a risk factor for OIIRR. However, the study design did not allow confirmation of the diagnosis of severe root resorption or blindness during the radiographic examination.[181]

Lee *et al*[181] found that dental invagination was not a risk factor. Regression analysis of various risk factors considering orthodontic root resorption has revealed a negative relationship for dental invagination. Mavragani *et al*[182] investigated the association between dental invagination and root shortening during orthodontic treatment and found invaginated teeth more often exhibited deviated root form which was considered a risk factor for EARR. However, invaginated teeth had delayed development and immature root which seemed to protect against root resorption.[147] They concluded that the mild form of dental invagination confined within the crown and not extended beyond the level of CEJ was not a risk factor for EARR.

4.7.1.2.2.10 Abnormal Root Morphology

The tendency of OIIRR was found to be greater in teeth with aberrant shaped roots.[11,45,49,151,178] Taithongchai *et al*[183] and Thongudomporn and Freer[180] reported that roots with short apices have enhanced root resorption. Levander and Malmgren[45] and Thongudomporn and Freer[180] found blunt-shaped roots frequently

showed root resorption when compared to normal root. However, Sameshima and Sinclair,[49] in a comparative study using radiographs taken before and after orthodontic treatment, reported blunted roots had the least resorption. This study concluded that teeth with other abnormal root morphology, for example, pipette-shaped, pointed, dilacerated and slender root, frequently showed ERR when compared to teeth with a normal root shape.

Recently, Oyama *et al*[184] investigated the stress distribution at the root apex with a variety of root shapes during orthodontic force application using FEM. They discovered that short, bent and pipette root shape resulted in a greater loading of the root than normal root shapes during orthodontic force application which suggested root deviations tended to promote root resorption.

One study compared 84 patients with at least one dental anomaly and 84 patients with no abnormalities.[181] The anomalies included agenesis, small or peg-shaped lateral incisors, dens invaginatis, taurodontism, ectopic eruption and short roots. Root lengths on radiographs before and after treatment were compared. They discovered that patients with dental anomalies were not at a higher risk of OIIRR.[181] Some studies have suggested that the tendency of EARR increased with increased root length.[49,178] This was due to longer teeth requiring stronger force to move, and the actual displacement of the root apex was larger during tipping or torquing movements. However, apical shortening that occurs in the shorter root seems to be of greater concern.

4.7.1.2.2.11 Root Resorption Prior to Orthodontic Treatment

Patients with pre-existing evidence of root resorption have been found to be at greater risk in developing further severe OIIRR with treatment.[44,159]

4.7.1.2.2.12 Previous Endodontic Treatment

Conflicting reports remain in the literature regarding the susceptibility of non-vital endodontically treated teeth to OIIRR. One group has found a greater incidence of OIIRR in endodontically treated teeth.[185] The increase in root resorption in the study appeared to be biased as the non-vital teeth were treated endodontically as a result of trauma.[185] In experimental animals, however, the incidence of root resorption following orthodontic treatment was the same for vital and non-vital teeth.[186,187] Spurrier *et al*[154] found vital incisors resorbed to a significantly greater degree than endodontically treated incisors. No significant differences were apparent between males and females when endodontically treated incisors were compared.[154] Many other authors believed that endodontically treated teeth were more resistant to root resorption due to an increase in dentine hardness and density.[7,60,154,178]

The disagreement among these studies may be due to the presence or absence of active inflammation related to residual infection during the orthodontic movement. Orthodontic tooth movement itself creates an inflammatory response that may increase an already existing resorptive process. A successful endodontically treated tooth with healthy periodontal support, in the absence of inflammation, should not be more susceptible to resorption than a normal tooth.

4.7.2 Mechanical Factors

Orthodontic tooth movement and biomechanics have been found to account for approximately a tenth to a third of the total variation in OIIRR.[52,152] One study showed up to 90 per cent of variation has been attributed to the extent of tooth movement.[156]

4.7.2.1 Duration of Treatment

Most studies agreed that the severity of OIIRR was directly related to the duration of orthodontic treatment.[45,63,145,149,165,175,188-190] Only a limited number of studies disagreed with this finiding.[166,179] Stenvik and Mjor[38] and McFadden *et al*[63] found that the longer the orthodontic treatment, the greater the degree of root resorption observed. Taithogchai *et al*[183] found a weak but highly statistically significant relationship between the amount of apical root shortening and the duration of active treatment. The degree of root resorption increased to 100 per cent of patients in active treatment for a period of seven years.[41]

Artun *et al*[191] evaluated standardised periapical radiographs of the maxillary incisors made before treatment (T1) and at about six and 12 months after bracket placement (T2 and T3 respectively) of 2467 patients. The risk of one or more teeth with more than 1.0 mm resorption from T2 to T3 was 3.8 times higher than the risk of one or more teeth with more than 1.0 mm resorption from T1 to T2.

Smale[50] radiographically assessed the amount of apical root resorption on average six months post initiation of fixed orthodontic appliance therapy. The results showed that root resorption began in the early levelling stages of orthodontic treatment.

About 4.1 per cent of patients studied had an average resorption of ≥1.5 mm of the maxillary incisors and about 15.5 per cent had one or more maxillary incisors with resorption of ≥2.0 mm from three to nine months after initiation of fixed appliance therapy.

In a randomised clinical trial study, Brin *et al*[176] compared the amount of EARR in treating Class II malocclusion with one phase and two phases of treatment. The results showed that 11 per cent of central incisors and 14 per cent of lateral incisors demonstrated moderate to severe, i.e. 2 mm, EARR. The proportion of incisors with moderate to severe EARR was slightly greater in the one phase treatment group. Significant associations existed among EARR, the magnitude of overjet reduction and the fixed orthodontic appliance treatment time.

4.7.2.2 Distance of Tooth Movement

As previously discussed, teeth that were moved large distances had extended exposure to the resorptive process. Therefore, the severity of OIIRR may be regarded to be positively related to the distance of tooth movement.[62,166,190,192,193] The upper incisors are commonly moved the greatest distance and are at the highest risk of OIIRR.[190,192-195]

4.7.2.3 Magnitude of Applied Force

Many animal studies[196-198] and human studies[13,39,199-201] have agreed that the force magnitude was directly proportional to the severity of OIIRR. Heavy force induces excessive hyalinisation and interferes with repair process of resorption craters.[6,38,39,68,197,202] King and Fischlschweiger[197] reported that light forces

produced more rapid tooth movements with insignificant cemental cratering whereas intermediate or heavy forces resulted in slower displacements and a substantial amount of root resorption. More recent studies on human upper first premolars involved 25 grams and 225 grams buccal force in an SEM study and 25 grams and 225 grams intrusive force in a micro-CT study found an increased amount of OIIRR with an increased force level.[14,16]

On the other hand, Owman-Moll *et al*[203,204] claimed that root resorption was not force dependent. Owman-Moll *et al*[198] compared the effects of two controlled, continuous forces of 50 cN and 100 cN on tooth movement and root resorption. The severity of root resorption, which was measured as the extension and depth of resorbed root contour and size of root area on histological sections, did not differ significantly when the applied force was doubled to 100 cN. Similarly, Owman-Moll *et al*[203] investigated the effect on tooth movements and root resorption when a fixed orthodontic appliance was activated with a controlled continuous force of 50 cN or with four fold larger force (200 cN). There was also no significant difference in frequency or severity between the two forces used.

4.7.2.4 Different Appliances and Treatment Techniques

Numerous studies have compared the extent of root resorption following treatment of different types of orthodontic appliances. Parker and Harris[156] compared the EARR among cases treated with the Tweed standard edgewise technique, Begg lightwire technique and Roth-prescription straightwire technique and found no difference among the techniques. Similarly, Beck and Harris[145] compared in-treatment root resorption between Begg and Tweed techniques and found no difference between the

techniques. In contrast, McNab *et al*[205] found more EARR in patients treated with Begg appliances than edgewise appliance. The excessive lingualisation of the maxillary incisor root by torquing force at the end of stage III of Begg technique may explain the higher incidence of EARR.

Blake *et al*[155] compared radiographically the amount of apical root resorption after orthodontic treatment with edgewise and Speed appliance. The Speed system provides a continuous rotatory and torque action through its spring clip mechanism in contrast with the edgewise appliance that may provide an interrupted force. There was no statistically significant difference in root resorption between the two appliance systems. On the contrary, a recent radiographic study showed that the Speed appliance caused more root resorption when compared to the Tip-Edge and MBT straight wire technique.[206]

Reukers *et al*[207] compared radiographically the prevalence and degree of apical root resorption after treatment with straightwire and standard edgewise appliances. There was no statistically significant difference between both groups at the end of active treatment. The mean prevalence of apical root resorption was 75 per cent for patients treated with straightwire and 55 per cent for patients treated with standard edgewise appliances. In contrast, Mavragani *et al*[64] showed significantly more apical root resorption of both central incisors in the standard edgewise appliance therapy than in straightwire edgewise groups. No significant difference was detected for the lateral incisors. This was possibly due to the less matured lateral incisors which tolerated root resorption more effectively.

Alexander[177] evaluated the differences in the extent of root resorption between continuous arch and sectional mechanics. Both treatment groups exhibited the same levels of resorption indicating that the side effect of treatment may be due to individual variation and not to the "round tripping" of teeth.

Janson *et al*[208] compared EARR after orthodontic treatment with three different fixed appliance techniques, standard edgewise technique, edgewise straight wire system and bioefficient therapy. The bioefficient therapy presented less root resorption than others. It was suggested to be attributed to the use of heat activated and superelastic wires, bracket design and the use of smaller rectangular stainless steel wire during incisor retraction and finishing stage as compared to the other techniques.

One radiographic study established that fixed appliances were more detrimental to the roots of upper incisors than removable appliances.[143] Eighty-one patients who were treated with removable appliances alone, activators and spring plates, were compared to 638 fixed appliance patients. There was a highly significant difference in the changes in root length. The maximum root shortening values were 0.39 mm for activators and 1.46 mm for spring plates. Another radiographic study by the same authors[61] involved a comparison of patients treated with full fixed edgewise appliances and patients treated with activators only. The activator group was found to have no OIIRR. They concluded that open activators, plates with clasps and vertical elastics had a very low correlation with root resorption. Patients treated with fixed appliances, rectangular arch wires and Class II elastics had notable OIIRR.

It has been suggested by one group of authors that the use of intermaxillary elastics increased the amount of OIIRR due to the existence of jiggling forces,[143] however, another group of authors found no correlation.[190]

Daimaruya *et al*[209] studied the effects of molar intrusion on the nasal floor and tooth root using a skeletal anchorage system in beagle dogs. The intruding premolars were resorbed at their apices and the root resorption partly reached into the dentine without the formation of reparative cementum. This was different from their earlier study that showed minimal root resorption with formation of new cementum found on mandibular intrusion.[210] This could be due to the compressive force against the nasal membrane under orthodontic force loading.

Kennedy *et al*[211] demonstrated that in children treated for Class I crowding, the roots of all teeth were 1 to 2mm shorter in patients who had fixed appliance treatment than in patients with serial extraction alone.

4.7.2.5 Direction of Force

The type and direction of tooth movement have a considerable role in OIIRR. It is expected that intrusion and torque have a higher force per unit area and thus cause more tissue necrosis and OIIRR.[7] Some authors suggested less root resorption associated with bodily movement compared with tipping due to the different stress distribution.[7,212] Reitan[68] has shown that ERR was weakly correlated to force magnitude (25 to 240 grams) and closely related to the type of tooth movement.

Han *et al*[213] compared the amount of root resorption in the same individual after application of continuous intrusive and extrusive forces using SEM. The study showed intrusion of teeth caused about four times more root resorption than extrusion. In addition, Weekes and Wong[214] observed root resorption at the interproximal region of the cervical third part of the root after extrusion indicating orthodontic extrusion was not without risk. Costopoulos and Nanda[215] investigated the effect of intrusion on EARR of maxillary incisors and concluded that intrusion with low forces could be effective in reducing overbite while causing only a negligible amount of EARR. McFadden *et al*[63] looked at retrospective data of 38 patients with deep bite, who were treated with utility arches to intrude incisors and reported an average root shortening of 1.84 mm for maxillary incisors and 0.61mm for mandibular incisors with no significant correlation between resorption and the amount of intrusion. Melsen *et al*[216] investigated the degree of root resorption after intrusion of incisors in adult patients with marginal bone loss. The results showed root resorption varied from 1 to 3 mm.

Jimenez-Pellegrin and Arana-Chavez[217] investigated the presence, location and severity of root resorption after orthodontic rotation for different lengths of time. SEM revealed many root resorption craters on all rotated teeth. The resorption areas were located mainly at the medial root third and in regions that corresponded to the prominent zones of the roots.

Casa *et al*[199] investigated the occurrence, localisation and extension of root resorption after fixed appliance treatment with a continuous torque force. The SEM analysis showed many resorption craters on the lingual side in the apical third of the roots. Resorption processes were also observed on the buccal root surface in the

cervical third of the roots. A higher magnitude of moments produced exposure of root dentine evidencing pronounced root resorption. Goldin[189] investigated the effect of labial root torque on the maxilla and incisor root apex. The overall apical root resorption was 12.7 per cent per year.

4.7.2.6 Duration of Force Application

There are conflicting reports as to whether continuous or discontinuous force produces a difference in the amount of OIIRR. A pause in tooth movement allows the resorbed cementum to heal which may produces less root resorption.[212,218-222] There were a number of studies with varying durations and frequencies of interruption in the applied forces which have led to varied results.

Reitan[7,223,224] advocated the use of intermittent forces to prevent the development of root resorption by allowing reparative processes to occur during periods with little or no force. This was confirmed by an animal experiment by Rygh and Brudvik[225] that has showed an association between the duration of the root resorptive process and the duration of force and presence of necrotic tissue in the PDL. Harry and Sims[39] suggested that duration of force was a more critical factor than magnitude.

Levander *et al*[219] radiographically evaluated the effect of a treatment pause of two to three months, on teeth in which EARR was discovered after an initial treatment period of six months with fixed appliances. The amount of root resorption was significantly less in patients treated with a pause than in those treated without interruption. The intermission of the forces facilitated reorganisation of damaged periodontal tissue and reduced root shortening. Maltha and Dijkman[220] compared

the amount of root resorption after continuous (24 hours per day) and discontinuous (16 hours per day) force application and reported more resorption in dogs when using continuous than intermittent forces. Acar *et al*[218] compared the effects on root resorption of continuous and discontinuous force application. The degree of root blunting was assessed by visual scoring on composite electron micrographs. It was discovered that the mean percentage of resorption affected areas was smaller and apical blunting was less severe under the discontinuous force. Konoo *et al*[226] examined and compared root resorption in rats following continuous and intermittent forces. The results showed the continuous force groups had more root resorption than intermittent force (activated for several one hour intervals) group. Weiland[222] compared the amount of root resorption when subjected to constant or dissipating forces. Constant force was induced by a superelastic wire for 12 weeks whereas dissipating forces was induced by stainless steel wire that was activated every four weeks. The volume of resorption craters was measured using 3D digital images made with a confocal laser scanning microscope. The results showed that the resorption craters on the teeth receiving constant force were 140 per cent greater than on the teeth of the dissipating forces group. Kameyama *et al*[227] examined the effects of inactive periods of force on the amount of root resorption during experimental tooth movement in rats. The area of root resorption in the four and nine hours inactivation per day groups was significantly less than that in the zero and one hour of inactivation per day groups. This was due to a decrease in mechanical stress and hyalinised tissue, recovery of form and function of the blood vessels, reduction of cytokine production and subsequent odontoclast formation.

Owman-Moll *et al*[228] compared the effects of continuous (24 hours per day) and interrupted continuous (interrupted one week every fourth week) forces in

adolescents and reported no difference in the amount or severity of root resorption. This was confirmed by Dermaut and DeMunck[62] in which the study concluded no significant relationship between the duration of the force and root resorption.

4.7.2.7 Extraction versus Non-extraction Treatment Protocols

There are studies which have discussed the amount of OIIRR associated with extraction treatments.[63,190,205] The approach of categorising an extraction or non-extraction plan as being associated with OIIRR is overly simplistic. Attention should be drawn to the distance the teeth are moved. Extractions for severe crowding do not have as much impact on movement of the maxillary incisors as the displacement following extractions for overjet reduction.

In the radiological study by Sameshima and Sinclair[190] involving 868 patients from six fixed edgewise practices, extractions and the extraction pattern were amongst the variables assessed and related to the severity of OIIRR. The authors found that patients who had four first premolars extracted had more root resorption than those patients who were treated with a non-extraction protocol. In addition, patients with other types of extractions including four second premolars, a mandibular incisor and asymmetric extractions also had more OIIRR than non-extraction patients. In contrast, cases which only involved extraction of the upper first premolars did not have more resorption than the non-extraction cases. They acknowledged that this contradicted their own conclusions that overjet and horizontal distance of movement of root apices are significant contributing factors to OIIRR.

4.8 Orthodontic Relapse and Orthodontically Induced Inflammatory Root Resorption

Following the active appliance removal, there is a conversion of the former pressure side of the active treatment period into the tension side during the relapse period.[229] Langford[9] showed that relapse forces were capable of causing significant root resorption for up to three months after RME. Zimring and Issacson[230] found that residual loads acting on the expansion appliance dissipated within five to seven weeks during retention. In addition, the teeth were held in an overcorrected position following expansion which resulted in an abnormal occlusal relationship. The occlusal force may be sufficient to perpetuate a small amount of ongoing resorption. Barber and Sims[8] argued against this concept as there was no significant resorption on the roots of extracted mandibular premolars which had opposed anchor premolars for up to 32 weeks of fixed retention. However, it has been recommended that retention with fixed attachments be applied with caution as occlusal trauma to the fixed teeth may lead to extreme resorption [231]

4.9 Clinical Consequences of Orthodontically Induced Inflammatory Root Resorption

A reparative process in the periodontium commences when the applied orthodontic force is discontinued or reduced below a certain level.[6,7] Therefore, the risk of tooth loss following orthodontic therapy is not high.[60,166,232]

A long term radiographic evaluation of root resorption after active orthodontic therapy revealed progressive remodelling of the root surface.[60] The jagged resorbed edges were smoothed and sharply pointed root ends were round with time. However, the original root contours and lengths were never re-established. Severely resorbed teeth were found to be functioning in a reasonable manner. Out of the 100 subjects that were studied, the worst outcome was hypermobility which were only observed in two patients.[60] Similarly, VonderAhe[166] examined 57 patients who had suffered from mild, moderate and severe amounts of OIIRR, only one case of hypermobility or other detrimental consequences were detected following 6.5 years of post-retention.

In a review article by Vlaskalic *et al*[149] six references were found from between the years 1914 and 1997 that discussed cases of EARR that caused a problem to the patient and the clinician. There were no reports found with tooth loss from severe apical root resorption after orthodontic treatment unless there was some other form of trauma to the tooth.

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 root length and intra-alveolar root length.[233] They found a significant risk of tooth mobility if an upper incisor had OIIRR resulting in 9 mm root length or less. Thus, they recommended regular follow-up assessments of teeth with severe OIIRR.[233]

A reduction in root length due to apical resorption has been described as less detrimental than an equivalent loss of periodontal attachment at the alveolar crest especially in cases ≤3 mm of early root resorption.[47] Kalkwarf *et al*,[234] with the aid of a computer graphics system, showed a nearly linear relationship between root length and percentage of periodontal attachment. Results indicated that 4 mm of root resorption translated into 20 per cent of total attachment loss and 3 mm apical root loss equals only 1 mm crestal bone loss. After the initial 2 mm apical root loss, calculations revealed every additional 2 mm root loss equaled to only 1 mm of crestal bone loss. Therefore, patients who were susceptible to marginal periodontal breakdown may have a higher risk of losing severely resorbed teeth prematurely. This emphasised the importance of periodontal disease control in patients with severely resorbed teeth. In addition, teeth with abnormally short roots and loss of periodontal attachment may not be suitable as future bridge abutments. Vlaskalic *et al*[149] warned that with the increasing number of adult orthodontic patients, the combined long-term effects of apical root resorption and alveolar bone loss may produce more negative sequelae than has been revealed by the studies to date.

The loss of root length during orthodontic treatment moved the centre of resistance coronally. Therefore, the same amount of torque on the tooth would have a greater effect than if the root were intact.[235] This may predispose the tooth to further root resorption.

Lee *et al*[236] investigated the different dental professionals' perceptions towards the significance of root resorption and found general dental practitioners were the most concerned about root resorption. The orthodontists attached significance to root resorption the least followed by periodontists and prosthodontists. The general dental practitioners viewed about 28 per cent of root loss as significant in comparison to around 32 per cent by the orthodontists. However, none of the groups found resorption levels of over 50 per cent was reasonable enough to extract and prosthetically replace the tooth.

4.10 Prevention and Management of Orthodontically Induced Inflammatory Root Resorption

Although little to no documentation showed OIIRR could result in tooth loss, other minor clinical consequences such as mobility, pulpal changes, periodontal attachment loss and future difficulty in restorations may occur. This could become a potential medicolegal issue, therefore, patient education, identification of at risk patients and preventive measures must be considered prior to orthodontic treatment.

Clinically, a number of approaches have been suggested in the literature to minimize OIIRR and were summarized by Vlaskalic *et al*[149] and Ghafari.[167] The recommendations included decreased treatment duration,[38,45] the use of light intermittent forces,[6,38,39,68] avoidance of sustained jiggling intermaxillary elastics,[61] limit tooth movement for OIIRR prone teeth e.g. intrusion and torque,[156] habit control[157] and a thorough assessment of familial tendency and medical history.[49,63,102,237]

Vlaskalic *et al*[149] also suggested that the treatment of moderate to severe malocclusions be commenced when most of the incisors had open apices, which was before the age of nine years. They recommended this strategy as incomplete root formation has been found to be significantly associated with a lower severity of OIIRR.[41,143,146]

It was strongly suggested that periapical radiographs should be taken at least every year to determine the presence of root resorption.[167] Minor resorption or an irregular contour of the root that were seen six to nine months after the last

radiograph indicated an increased risk of further root resorption.[45] The original treatment goals must be reassessed depending on the extent of root resorption detected. The results compromised relative to the amount of root resorption was acceptable or at least the force levels should be modified or a two to three month pause in treatment with passive arch wires should be implemented.[219] Additional x-rays should be taken every three months in at risk patients to monitor the progress of root resorption.[11,167] The amount of additional tooth movement required should be considered against the amount of root resorption acceptable, that is, no more than one third of the root length. It was mandatory to take final radiographs at the time of removal of fixed appliances.[55] In the case of teeth with severe OIIRR, follow-up radiographs were recommended until additional root loss was no longer detected.[233]

Numerous animal studies have been conducted to investigate the possibility of reducing the risk of OIIRR by applying drugs that modulate the activity of osteoblasts, osteoclasts and odontoclasts. Arginine-glycine-aspartic acid containing peptides inhibit the resorptive activity of isolated clast cells by targeting the intergrin receptor expressed by odontoclasts and have shown to be effective in reducing root resorption during tooth movement.[238] Low dose systemic administration of doxycycline in rats may have an inhibitory effect on OIIRR via reduction of odontoclasts, osteoclasts, mononuclear cells and TRAP positive cells on root.[239] Low doses of thyroid hormone also played a protective role on the root surface against OIIRR.[240] Steroid treated rats also displayed significantly less root resorption on the compression side and fewer TRAP positive cells within the PDL space on the same side.[119] However, many of these drugs also altered the activity osteoblasts and osteoclasts in alveolar bone which may interfere with the rate of

tooth movement. On the other hand, PGE_2, enhanced orthodontic tooth movement but increased the amount of root resorption.[3,241]

Recent research has focused on identifying biological markers in the gingival crevicular fluid (GCF) in the light of relating these markers and the risk of OIIRR. If successful, this technique could be easily implemented in identifying the patients at risk of OIIRR prior to orthodontic treatment and treatment planning could be modified accordingly. Mah and Prasad[242] showed elevated levels of dentine phosphoproteins in the GCF in resorbing primary teeth and active orthodontic treated teeth compared to untreated permanent teeth. In addition, Balducci *et al*[243] identified an increase in dentin phosphophoryn and dentin sialoprotein concentration in the GCF of the severe root resorption group. Therefore, dentin phosphophoryn and dentin sialoprotein could be suitable biological markers for identifying at risk patients and monitoring root resorption during orthodontic treatment.

5. Repair of Orthodontically Induced Inflammatory Root Resorption

Repair of root resorption craters began when the applied orthodontic force was discontinued or reduced below a certain level.[6,7] According to Schwarz,[244] when the orthodontic force reduced below the optimal force of 20 to 26 g/cm^2, root resorption stopped. The reparative process may be seen simultaneously with the resorption process.[8,38,39,245] Many studies have demonstrated that the resorptive defects were repaired by deposition of new cementum and re-establishment of new PDL.[8,9,68,246-248]

There were numerous studies on the repair of OIIRR, many of which were animal studies. However, results from the experiments in lower animals, for example, rats and rabbits, were not valid for humans because their teeth continued to erupt with cementum being formed throughout their life time. In addition, the difficulties with the use of higher animals such as monkeys in studying root resorption required adequate sample size which could be costly. All human studies available involved extracted teeth which may have lost part of the PDL and the response of the periodontium could not be studied. Therefore, the repair of OIIRR remained poorly studied.

5.1 Types of Repaired Cementum

Henry and Weinmann[43] defined two types of repair. Anatomic repair was characterised by the restoration of the root surface to its original contour and a functional repair occurred when the exposed dentin has been covered by a thin layer of repair cementum, resulting in a deficient root outline. In both types, the PDL was restored to its original width.

Vardimon *et al*[229] studied 87 adolescent monkeys which were subjected to palatal expansion and reported that the majority of root resorption was repaired partially. The study identified two forms of partial ERR repair following four months of retention after RME. They were non-functional retarded repair cementum, also referred to as arrested resorption, and a functional rapid repair cementum. Non-functional retarded repair cementum was depicted as an absence of Sharpey's fibres and slow deposition of reparative cementum with ill-defined separation between narrowed sequential incremental lines. In contrast, functional rapid repair cementum was illustrated by detectable Sharpey's fibres. This type of repair was usually found in superficial ERR lesions and in moderate EARR sites. There was an early lag phase in this type of repair where no cementum was deposited. The extent of the lag phase can be explained by the dissipation of residual forces[249] and the replacement of clastic cell population by a blastic cell line.[38,68]

5.2 Pattern of Orthodontically Induced Inflammatory Root Resorption Repair

5.2.1 Temporal Pattern of Orthodontically Induced Inflammatory Root Resorption Repair

Numerous studies have been documented regarding the time of onset of root resorption repair. Root resorption repair was recorded as early as the first week of retention.[10] Filho *et al*[250] suggested cementum repair following root resorption was likely to occur within two to three weeks if the affected surface was not very large. Stenvik and Mjor[38] and Reitan[68] also noted a delayed repair response of 35 to 40 days after force termination. Longer delay has also been documented by Harry and Sims[39] where the appearance of cellular repair cementum in the apical regions occurred at 70 days. Barber and Sims[8] found no periodontal reattachment even after 36 weeks of retention subsequent to RME treatment. The process of ERR continued even during extended retention periods of up to one year.[8,9,70] This was also highlighted in Reitan's[68] study of orthodontic procedures in dogs which found that once root resorption was started by strong continuous orthodontic forces, much lighter pressure could maintain or increase the resorptive process.

The amount of root resorption repair increased with time.[8-10,203] Owman-Moll and Kurol[251] demonstrated more reparative cementum in the resorption cavities after six and seven weeks of retention when compared to two and three weeks of retention. The reparative process increased during the first four weeks of retention and after five to six weeks, the process slowed down and reached a steady phase.[10] On the other hand, Vardimon *et al*[229] reported that the healing process in monkeys reached a

steady phase after 42 to 56 days. Langford[9] showed that active resorption slowed significantly after about three months of retention and the resorptive defects were repaired over the retention periods of 14 to 52 weeks. The reparative process seemed to continue for a long period of time. Timms and Moss[70] found areas of resorption and repair in the apical third of the teeth extracted after retention periods of 21 to 23 months. Ongoing repair gradually restored the resorption defects, however, the repair remained incomplete even a year after expansion.

Vardimon *et al*[229] 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. This was followed by a peak phase (14 to 28 days) which was a spurt in matrix formation. The peak phase was characterised by an initial incorporation of extrinsic fibres into the intrinsic cementum matrix which suggested a development towards functional repair. Then, a steady deposit phase (42 to 56 days) of mixed fibrillar cementum followed which was interrupted by the removal of the retention appliance.

5.2.2 Spatial Pattern of Orthodontically Induced Inflammatory Root Resorption Repair

Different spatial patterns of repair have been described, starting either from the periphery of the resorption cavity[5,13,252,253] and or from the centre of the resorption crater outward.[8]

Langford[9] showed repair commenced centrally within the resorption craters and expanded peripherally with new reparative tissue. However, concomitantly,

resorption continued to spread at the periphery of the resorption site.[8,9] This could theoretically be explained by the fact that the squeezing of tissue between the tooth and bone surface was less pronounced on the bottom of the resorption cavity than in the periphery due to the resilience of the tissue.[251] Consequently, cell proliferation and regeneration of tissue were more pronounced in the deep central part of the resorption cavity.

On the other hand, Brudvik and Rygh[5] found that the reparative process started in the periphery of the root resorption craters while active resorption was still occurring beneath the centrally located over-compressed hyalinised zone. This was agreed later by Faltin[13]. In addition, a SEM study on the post-mortem orthodontically treated tooth material showed the resorption area was characterised by the co-existence of centrally located active resorption processes and reparative activity in the marginal zones.[254]

Sismanidou and Lindskog[255] agreed with both Langford[9] and Brudvik and Rygh[5] and mapped two types of spatial repair patterns of orthodontic surface resorption. Cementum deposition proceeded either from the periphery or starting in the centre of the resorbing areas, although the deposition starting centrally was argued to be artifactual. Owman-Moll and Kurol[251] revealed in their study that healing cementum only occurred centrally in the bottom of the cavity and the central peripheral on one of the lateral walls of the cavity. In partial repair, healing cementum was seen more often in the central part of the resorption crater than central peripheral areas of the crater.[251] Reparative tissue was never observed on the lateral walls alone.

5.3 Location of Orthodontically Induced Inflammatory Root Resorption Repair

The type of treatment dictated the location of root resorption and repair. A histologic report has provided some light microscopic data on the distribution of RME-induced root resorption and repair after two years of retention.[70] The resorptive defects were generally observed mesiobuccal and distobuccally. Repair by "osteoid" tissue predominated in the more coronal resorption craters of teeth extracted two years after RME and continued resorption was observed simultaneously in the apical thirds of these teeth. This was in concordance to the SEM study by Barber and Sims[8] where results showed healing in the cervical zone while resorption continued in the apical zones. This could be attributed by the deficiency of Sharpey's fibres in cellular cementum which made repaired and apical cementum more vulnerable to resorption.[256]

In a study where buccally directed force was induced and retained by a passive sectional arch wire during the retention period, resorption craters with secondary cementum were distributed with almost the same frequency in the cervical, middle and apical parts of the root.[10] Partial repair was most often seen in the cervical third of the root and anatomic repair in the middle and apical third. Anatomic repair was seen nine times more often in the apical third compared with the cervical third of the root.

5.4 Cellular and Acellular Cementum Repair

Unlike animals where root resorption is predominantly repaired by acellular cementum,[257,258] the repair material in humans is principally cellular cementum. This is because only the CIFC can fill a resorptive defect in a reasonable period of time in humans.[259] In cases of rodents, AEFC can manage to repair the resorptive defect rapidly because cementoblasts in rodents have a relatively high level of activity.

Henry and Weinmann[43] observed the autopsy material of 15 human's dentition with repaired resorption areas exhibiting many combinations of cellular and acellular cementum. Sismanidou and Lindskog[255] proposed that healing was initiated by acellular cementum formation and the pattern of formation changed gradually favouring a slow deposition of cellular cementum at more advanced stages of healing. Therefore, acellular cementum was usually seen at the bottom of the resorption crater and with time it was covered by cellular cementum.

Similarly, in a monkey model experiment, Vardimon *et al*[229] found attenuated apposition of acellular cementum in the bottom of the resorption crater during the onset of resorption. The deceleration in repair cementum formation during the relapse period was characterised by an increased formation of extrinsic fibres in mixed fibrillar cellular cementum. This was confirmed by Bosshardt[260] who suggested that the initial reparative cementum was often of an acellular type but the continued repair process of the resorption crater occurred with rapidly forming cellular cementum. Recently, a post-mortem human study revealed root resorption craters were predominantly refilled with CIFC. Only a few craters were lined with

a thin coating of AEFC so that complete anatomical and functional restorations of periodontal structures were demonstrated. Therefore, repair has been claimed to be a mixture of cellular and acellular cementum and in some instances even with osteoid tissue.[70]

The OIIRR in young individuals is repaired almost exclusively with cellular cementum.[10] Cellular cementum repair is readily recorded in young individuals in whom regeneration processes are faster and cells are more easily captured by growing repair tissue.

5.5 Degree of Root Resorption Repair

The physiological resorption craters appeared as shallow, irregular concavities, not exceeding 0.23 mm in depth and 0.85 mm in length.[259] These were undetectable radiographically.[259] Aguilar *et al*[142] compared the repair of physiological resorption craters in young and elderly patients. Young patients displayed 30 per cent of root resorption and 19 per cent of repair while older patients showed 94 per cent of root resorption and 41 per cent of repair.

Fritz *et al*[254] investigated post-mortem human material of a 24 years old orthodontically treated male. Seventy-five per cent of the lateral root resorption craters showed marked reparative dynamics. The length of repaired defect averaged 531±541 μm (56.6 per cent) and repaired height averages 58±41 μm (28 per cent).

Owman-Moll *et al*[10] documented the amount of root resorption cavities repaired at different retention periods following six weeks of light buccally directed orthodontic force of 50 cN. After the first week of retention, 28 per cent of the resorption craters showed some degree of repair. The repair rose to 75 per cent after eight weeks of retention. Partial repair was recorded more often (17 to 31 per cent) than functional or anatomic repair during the first four weeks of retention. After five to eight weeks of passive retention, functional repair dominated the repair process (33 to 40 per cent). Resorptive areas with anatomic repair were registered six times more often after eight weeks of retention (12 per cent) than they were after the first week of retention (2 per cent). In a later study, Owman-Moll and Kurol[251] found 38, 44 and 82 per cent of resorption craters repaired after two, three and six to seven

weeks of retention respectively.

5.6 Biological Process of Root Resorption Repair

It has been assumed that if force was discontinued, active root resorption would cease.[6,7] However, Brudvik and Rygh[5] have shown that root resorption continued in hyalinised tissue even after active force has been terminated. A reparative process in the periodontium commenced after the applied orthodontic force was discontinued or reduced below a certain level.[6,7] It was hypothesised that the determinants of continued resorption and repair were associated with the persistence and removal of the hyalinised tissue.[5]

5.6.1 Physiological Root Resorption Repair[259]

Bosshardt and Schroeder[259] studied the mechanism of cementum attachment during physiological repair in human teeth. Following the resorptive phase, the odontoclasts withdrew from the resorbed root surface. The bottom of the Howship's lacunae was lined with a 1 to 2 μm thick layer of residual collagen fibrils which represented demineralised, residual dentinal matrix. A class of mononuclear cells was closely adapted to this matrix. These mononuclear cells produced a collagenous fibre fringe which was intermingled with the collagen fibrils of the residual dentinal matrix. There were two different modes of matrix production. The initial repair matrix was usually produced by fibroblast like cells and resembled that of the early AEFC. These fibroblast-like cells formed shallow surface indentations that were filled with bundled collagen fibrils. Advanced fibril formation and bundling usually resulted in an established dense fibre fringe oriented perpendicularly to the bottom of Howship's lacunae. Infrequently, typical

cementoblasts formed directly onto the resorbed root surface, a matrix similar to that observed during CIFC initiation. The collagen fibrils of this layer were randomly oriented with only occasional bundling. Focal mineralisation centres appeared within the initially produced collagenous matrix and accumulated at the external front of matrix obscuration. Further repair matrices were produced by cementoblasts and resembled CIFC. The newly produced collagen fibrils intermingled with those of the residual matrix before a basophilic and granular, electron-dense material obscured this junctional zone i.e. the future dentinocemental junction.

Three different cell types were seen in the resorption craters, osteoclasts and two types of mononuclear cells, one in close proximity to the resorbed surface and the others remote from the root surface. These latter cells had some characteristics of epithelial cells, although a basal lamina could not always be detected. Epithelial cell clusters in the root-related part of the PDL were commonly referred to as ECRM.

5.6.2 Orthodontically Induced Inflammatory Root Resorption Repair

Kurihara and Enlow[261,262] examined the periodontal attachment to remodelling alveolar bone in rats. They identified three types of periodontal membrane to bone attachments: adhesive, continuous and a composite of both types of attachments. One photomicrograph of a tooth subjected to experimental orthodontic forces showed an adhesive periodontal attachment to a resorbed dentine surface.

Bosshardt *et al*[263] analysed the structural and partial biochemical nature of the

interface that develops between resorbed dentine and repair cementum using human deciduous teeth as a model. The odontoclasts preconditioned the dentine matrix such that the reparative cementum became firmly attached. Bone sialoprotein and osteopontin first occupied the most accessible matrix elements and odontoclasts preconditioned the peripheral dentine matrix such that it was capable of accommodating non-collagenous proteins.

Sismanidou and Lindskog[255] examined the reparative process of human premolars that were subjected to buccally directed force and retained for different time frames. SEM showed cementum repair was manifested by micro-calcospherites of mineralised material in the resorption cavity and later by lacuna like depressions in the mineralising front indicative of cementocyte embedding. After 13 days of retention, there were extensive resorbed areas with some cementum deposition occurred predominantly in the centre of the resorbed areas covered by deposits of cementum with a granular mineralising front. By 20 to 25 days, cementum repair extended to the periphery of the resorbed areas. Some initial cementocyte embeddings were evidenced in the central part of the resorbed areas but patent dentinal tubules predominated in the resorbed areas. At 36 to 46 days, most resorbed areas showed signs of repair with most of them covered with reparative cementum in a continuous layer with the peripheral original cementum. Larger mineral globules were observed on the forming cementum surface and the entire surface of the reparative cementum was covered with minute cavities probably from the dissolved cementocytes. After 56 to 86 days, the reparative process progressed to involve all resorbed areas. Resorbed area could be seen within reparative cementum formation. At 160 days, no active resorption could be seen and continuous reparative cementum coverage was present which completely obscured

the resorption craters. All reparative cementum surfaces were continuous with the surrounding original cementum.

Vardimon *et al*[229] found in some cases that the functional repair in the apical area was characterised by hypercementosis. The overproduction of cementum generated side effects in the form of occlusion of the apical foramen, exostosis of cementum and displacement of the pulpal tissue toward the tension side.

5.6.3 Light Microscopy of the Repairing Orthodontically Induced Inflammatory Root Resorption

Light microscopic examinations of the effects of palatal expansion on human anchor teeth reported by Moss,[264] Rinderer,[265] Timms[266] and Timms and Moss[70] showed resorption and evidence of repair. However, there was no demonstration that attachment of periodontal fibres had occurred or was occurring in the area of resorption. In contrast, Langford and Sims[9] observed that the reparative cellular cementum appeared histologically and topographically identical to normal apical cellular cementum. The repaired cellular cementum tissue within areas of resorption displayed fibre bundles that were continuous with the periodontal fibre plexus adjacent to the root surface. There was no "clear zone" associated with periodontal fibre attachment at the cellular cementum fibre interface. This could possibly be explained by the adhesive type of attachment, similar to that described by Stahl *et al*[267] and Kraw and Enlow.[268] The orientation of the periodontal fibres associated with the more advanced repair areas, where the root contour was usually re-established, appeared to be mainly parallel to the normal root surface.

Brudvik and Rygh[5,253] have shown that the OIIRR reparative process was similar to cementum formation during tooth development which started in the periphery of the resorption craters. Root resorption occurred even after active force was terminated. This could be explained by the slowness of clast cells to adapt to a change of stimuli,[269] the persistence of necrotic tissue in the PDL enhanced root resorption and the retention of the tooth in the new position prolonging passive stress in the PDL. Osteoclastic activity decreased when all necrotic tissue in the PDL has been resorbed.

The PDL was compressed to approximately 25 to 60 per cent of normal size after two to seven days of experiment.[5] The PDL width then increased after ten days with or without persisting active force and 14 days after active force had ended. By 21 days, the periodontal width returned to normal size. A thin layer of cementum-like material, stained relatively dark by Haematoxylin and Eosin (H&E), was observed in the periphery of resorption craters at a distance from the hyalinised tissue. The multinucleated TRAP-positive staining cells concentrated between remnants of hyalinised tissue in the periodontal membrane and in root resorption craters. TRAP-negative mononucleated fibroblast-like-cells were observed close to the newly formed cementum. As the reparative process progresses, there was a reduction in the extent of root resorption and the number of TRAP-positive cells on the root surface and in the periodontal membrane. It was not infrequent to find ongoing root resorption without corresponding bone resorption. New PDL cells, fibres and blood vessels were observed where the hyalinised tissue had been completely removed. In the most peripheral areas of the resorption craters, regularly arranged fibres could be found from the root surface toward the middle of the PDL, while fibres were irregularly orientated in the resorption craters adjacent to

the central hyalinised zone. Root surfaces beneath the persisting hyalinised zone were not covered by new tissue.

5.6.4 Scanning Electron Microscopy of the Repairing Orthodontically Induced Inflammatory Root Resorption

One of the earliest SEM examinations on OIIRR repair was carried out by Barber and Sims.[8] The anchor premolars extracted after a short retention following RME revealed evidence of significant repair with continuing resorption. Interestingly, in this study, the longer a tooth was held in an overcorrected position during retention, the more comprehensive the resorption. This was refuted by Sismanidou and Lindskog[255] in which the study did not report an increase in resorption areas during retention. The resorbing Howship's lacunae had a granular appearance with the onset of remineralisation. At higher magnification, the initial mineral front, including the granular projections, had a coral like appearance. As remineralisation progressed, the Howship's lacunae on the floor of the resorption cavity were the first to be obliterated. The root contour was re-established by the filling of mineralisation in the resorption craters. Some defects were over-contoured, while others remained as shallow depressions.

The forming and resting mineral front was characterised by the presence of scattered cementocyte lacunae with the maximum diameter of 15 μm.[8] As cementocytes became more deeply embedded within the mineral front the surface openings became progressively smaller. Small groups of Sharpey's fibre depressions were occasionally found within the forming mineral front of repaired cementum. These fibre depressions with a surface diameter of approximately 2 to 10 μm were located centrally in the resorption crater floor.[8] Langford and Sims[9] found very few

Sharpey's fibre depressions in the repair tissues. The depressions generally occurred in rapidly mineralising tissue at the advancing periphery of repair areas. As repair progressed outward, the depressions may be mineralised as the fibres become incorporated as cementum matrix. Various diameters of fibres and fibre bundles entered the reparative tissue. The inserting fibres appeared to be continuous over the entire repaired cementum surface. At their junction with the repaired cellular cementum these fibres became indistinguishable from the repaired cellular cementum matrix.

Mineralisation within the repairing resorption craters was identified by a fragmented mineral front.[8] Continued mineralisation was characterised by fusion of the fragmented mineral front together into a coherent mineral mass. Resting mineral fronts of repaired resorption craters were mineral surface free from discrete particles. If particles were present, they coalesced with others into a confluent mass. There were two types of resting mineral front. One type of mineral surface appeared as a mat-like array of fibres and fibre bundles. The other type of the mineral surface was smooth, amorphous and featureless and most often observed in relation to mounds formed by an exuberant repair response. The forming mineral front of repair cementum was found to be topographically similar to the forming foetal bone[270] whereas the resting mineral front was indistinguishable from resting adult lamellar bone.[271,272] The difference was thought to be the result of variation in the rate of matrix production.

Similar to Barber and Sims,[8] Sismanidou and Lindskog[255] found two types of repair matrices with different mineralisation rates. The high rate of mineralisation was characterised by smaller roughly spherical mineralising patches and an apparent

random orientation of the organic matrix while the other type showed a regular and structured mineralising front within the collagenous matrix fibres.[8,270,271,273] The latter type was observed more frequently with longer retention times. This has been shown to be intrinsic fibre cementum,[271] forming at a relatively slow rate, while the initial microcalcospheritic mineralisation pattern has been suggested to be the result of "mineralisation within a thin layer of ground substance deposited after the cessation of active resorption".[8]

Hellsing and Hammarstrom[258] studied the hyaline zone and associated root resorption after orthodontic treatment of the upper first molars of rats. The orthodontic treatment consisted of a fixed buccal expansion appliance with an initial force of 250 mN. There was one week of appliance activation followed by appliance removal. SEM showed healing of the resorption craters by reduction in width as well as in depth. The surface of the reparative cementum had an even, glossy structure. Six weeks following removal of the appliance, a depression in the root surfaces remained. In the repaired resorption cavities, no dentinal tubuli was found which suggested that root resorption associated with the elimination of the hyaline zone was followed by formation of reparative cementum which prevented further communication between the pulpal and periodontal tissues.

Lindskog *et al*,[252] reported healing of the exposed dentin on the root surface subsequent to colonisation by cementoblast like cells from the periphery of the denuded area. Consequently, initial deposition of reparative cementum would take place from the periphery towards the centre of the exposed dentin areas.[252,274] This indicated that cementoblasts required a solid surface for migration[252] and the main source of cementoblast repopulation was located in the cell layers immediately

adjacent to cementoblasts surrounding the denuded dentin surfaces.[275] Nakane and Kameyama[276] noted rapid repair of resorbed dentine by cementoblasts derived from the PDL.

5.6.5 Transmission Electron Microscopy of the Repairing Orthodontically Induced Inflammatory Root Resorption

Brudvik and Rygh[253] studied extensively the ultrastructure of the OIIRR repair tissues in rats. The transition of active root resorption into a process of repair which occurred even in the presence of a light force was associated with the invasion of fibroblast-like cells from the periphery into the active root resorption site.

During active force application, multinucleated odontoclast-like cells with ruffled borders and clear zones indicating active resorption and fibroblast-like cells rich in rER and dense bodies were observed in the same resorption crater. This suggested a transition from resorption to repair. Fibroblast-like cells seemed to invade the craters from the periphery while active resorption by multinucleated odontoclast-like cells occurred in the central parts. This was in concordance with Lindskog *et al*[252] who reported the repair of surgically induced root cavities on the replanted teeth of monkeys was associated with migration of fibroblast-like cells from the periphery of the cavities.

In the later phase, more advanced repair was indicated by apposition of new cementum in some areas while the root dentine was covered with only a few fibrillar structures in the other areas. The repair process was similar to the early cementogenesis during tooth development.[277,278] New mineralised cementum was

observed on the resorbed root surface by 21 days. After deposition of the new cementum, the structures of a new PDL returned to normal. This finding correlated with their previous light microscopic studies.[5,253]

Different types of PDL cells have been identified near the root surface and at some distance from the resorption craters. Despite the difference in the morphology, the cytoskeletons of these cells were similar. Although some cells nearest to the resorption crater showed plump cuboidal shaped nuclei and resembled cementoblast-like cells during cementogenesis.[278] They could on the other hand be fibroblasts being seen in different planes of sectioning. During the initial phase of re-establishment of the new PDL, the cells near the resorbed root surface lacked preferential orientation.[5]

The multinucleated cells which occasionally appeared near the resorbed root dentine might be inactive odontoclast-like cells or a version of multinucleated cells being seen in the PDL during the removal of necrotic tissue.[69] The multinucleated cells were not involved with phagocytic activities due to their absence of vacuoles in the cell cytoplasm. They could be multinucleated fibroblast cells formed by fusion of adjacent mononucleated fibroblasts or lobulated mononucleated cementoblasts or fibroblasts.[279,280]

The presence of many dense bodies in the intra- and extracellular compartments indicated an early phase of cementum or collagen regeneration. These dense bodies may represent secretory granule products which transported procollagen molecules to the extracellular space through exocytosis.[281] The collagen on exposed dentine surfaces may be formed extracellularly in the areas where the extensions of fibroblast

cytoplasm were observed.

Cho and Garant[282] observed that cementoblast progenitor cells exhibited undeveloped rER prior to differentiation to cementoblasts in the dental follicle of developing roots in rats. The TRAP negative cells in resorption craters could be cementoblast progenitor cells. These cells differentiated into active form of AECF forming cementoblasts with developed rER in the resorption craters. This indicated that the new formation of AEFC was prepared in resorption craters immediately after resorption by odontoclasts.

A thin light junction zone was observed between the resorbed root surface and an electron dense zone where fibrillar structures were obscured. This was similar to the regeneration of cementum and PDL tissue attachment on surgically exposed root surfaces[246,283,284] and the repair after experimentally induced root resorption in rats.[285] The light zone was in areas without Sharpey's fibre insertions and not where fibres inserted into a new cementum. This zone indicated an initial stage of cementum formation which interfaced between old and new bone.[261] It was comparable to the dense granular material that contributed to a collagen free zone of cementum matrix. After more Sharpey's fibres had inserted into the new cementum, no light zone was observed which suggested a more intimate union of new and old mineralised root substance through the interlacing of the matrix component.[284,286]

Casa *et al*[287] documented a TEM study on root resorption following orthodontic torque movement. Some regions on the pressure sides displayed signs of repair after four weeks of torque movement. Similar to previous studies,[5,253] concomitant tissue damage and resorption of the root surface were observed. Some deeply

resorbed craters contained one or two large multinucleated TRAP positive clastic cells but not in contact with the root surface. Some cementoblast like cells were interposed between clastic cells and the root surface. Cells and matrices of PDL contained in these craters displayed many synthesis organelles. In several craters, cementoblast like cells were observed over a newly secreted organic matrix. Complete repair usually did not occur when dentine was resorbed. Repair of cementum occurred during continuous or intermittent orthodontic forces on human teeth and the repair process did not necessarily depend on the magnitude of the force.[13]

Faltin *et al*[13] studied the ultrastructure of the cementum and PDL changes after continuous intrusion with two different controlled forces in human teeth. Areas of tissue repair were evident in the cementum and PDL even with the maintenance of mechanical stress. The presence of numerous cells in diverse stages of differentiation had similar characteristics to those during cementogenesis. Rounded cementoblasts with their nucleus containing loose chromatin and obvious nucleolus as well as voluminous cytoplasm with well-developed rER cisterns, secretion granules and mitochondria were observed near the cementum. These cementoblasts were at different stages of differentiation establishing intercellular junctions and membrane inter-digitations between them. These cementoblasts also had an active process of production and secretion of collagen matrix that corresponded to the intrinsic fibres of the cementum.[259] There was also presence of Sharpey's fibres and an organised and newly synthesised non-mineralised cementum in these areas. Fibroblasts of the PDL were also observed at different stages of degeneration. The degeneration process seemed to limit the areas of altered extracellular matrix. Active fibroblasts were observed with their normal characteristics suggesting

concomitant repair of the involved PDL. In the irregular surface of resorbed cementum, an electron-opaque cement line was observed to which collagen fibrils appeared anchored. These findings were indicative of remineralisation and fibrillar re-attachment in the cementum.[82,262]

5.6.6 Immunohistochemistry of the Repairing Orthodontically Induced Inflammatory Root Resorption

Sismanidou *et al*[288] studied the resorption and regeneration of periodontal tissue in relation to orthodontic tooth movement and found the presence of KP 1^+ mononuclear cells located at a distance 50 to 100 μm from the root surface and multinucleated cells in resorption craters in close contact with the root surface. The mononuclear $KP1^+$ cells in the PDL may represent either precursors to odontoclasts or phagocytic scavenger cells of the macrophage lineage. The subsequent healing of the resorption craters was characterised by re-establishment of nervous, vascular and epithelial tissues as evidenced by $S\text{-}100^+$ filamentous delicate structures, factor $VIII^+$ vessels and $cytokeratin^+$ clusters of cells, respectively. Cementoblasts located in the vicinity of resorption craters, especially healing ones, appeared to show an up-regulation of epidermal growth factor receptors.

Jimenez-Pellegrin and Arana-Chavez[289] investigated the presence of osteopontin during cementum repair after rotational movement using high resolution immunocytochemistry. The experimental teeth were subjected to 25 grams of rotational force for two, three, four or six weeks. The results showed that both clastic cells and the root surface were immunolabelled for osteopontin. Therefore, osteopontin played a role in both cementum resorption and repair.

5.7 Factors Influencing the Resorptive-Reparative Process

Vardimon *et al*[229] suggested that the resorptive-reparative processes were regulated by three principles: ERR level of irreversibility, delayed resorption response and jiggling.

ERR level of irreversibility refers to resorption damage at a level above which no repair could take place. According to Vardimon *et al*,[198] OIIRR magnitude was assigned as 0 (no ERR), 1 (cemental and superficial dentinal ERR), 2 (augmented dentinal ERR, where the total unresorbed surface area exceeded the total resorbed surface area), 3 (severe dentinal scavenging where the total resorbed area was greater than the unresorbed area). An ERR score of 2 would undergo repair unless there was continuing damage due to a delayed resorption response. Resorbed sites with an ERR score of 3 were irreparable.

Delayed resorption response was responsible for the lag in the repair response. The resorption process started at day 10 to 20 from the onset of forces[8,9,38,67] and continued for several weeks after force elimination.[39,70,212] Increased time and severity of the active resorption phase could extend the delay phase.

Jiggling resorption was related to oscillating tooth movement. Jiggling resorption might lead to a delayed auto-immune response,[290] and or a boost in odontoclast by recurrent bone/tooth contact and or motility of existing osteoclasts. It was explained by the conversion of osteoclasts from the previous compression side to the new compression side.

Owman-Moll *et al*[10] documented a large individual variation in repair of root resorption with no association between right and left sides in each individual.

The ECRM have been suggested as a factor in protecting the root surface.[72,274] Damage to these cells during orthodontic tooth movement may inhibit its capacity to protect the root against resorption. Another main barrier against root resorption seemed to be the cells that covered and supported the superficial layers of the root cementum[291] and unlike osteoblasts were not affected by PTH.[292] Unmineralised and mineralised cementum itself may be an important factor in preventing resorption.[71]

Harry and Sims[39] showed that repair initiation was not a simple result of force magnitude and duration. Environmental differences in the anatomy and biochemistry of the ligament which accompanied varying tooth movements resulting from continuous or intermittent pressures influenced the times and sites of cellular cementum formation. Repair occurred at the pressure area when intermittent forces were applied because forces decreased permitting the PDL to recover before a new activation. On the contrary, Bonafe-Oliveira *et al*[293] reported signs of repair in previously resorbed cementum following the application of continuous force on rat molars. This was in agreement with the human study performed by Faltin[13] with continuous intrusive forces.

Cementoblast activity was influenced by growth factors such as epidermal growth factor, transforming growth factor – β, insulin like growth factors (IGFs) and others.[288] IGFs were believed to behave as proliferative factors for PDL cells,[294] HERS[295] or ECRM.[296] Gotz *et al*[297] investigated the role of IGF when the teeth

were subjected to nine days of force. Orthodontic force seemed to upregulate IGF-I and to a lesser extent IGF-II. Reparative cementum contains IGF-binding proteins-3 and -6. These findings indicated that the IGF system besides other growth factor families played a role in the repair process. Sismanidou *et al*[288] showed upregulation of epidermal growth factor during repair.

El-Bialy *et al*[298] investigated the effect of low-intensity pulsed ultrasound (LIPUS) on root resorption. SEM showed a statistically significant reduction in resorption area and the number of resorption craters in LIPUS exposed premolars. Histology showed healing of some resorbed root by hypercementosis. All lesions repaired anatomically i.e. original root contour was established with repaired cementum. Results suggested the use of LIPUS during orthodontic treatment to enhance repair of root resorption with continuous orthodontic force. The increased formation of reparative cementum that covers most resorption craters suggested the direct stimulatory effect of LIPUS as cementogenesis.

5.8 Epithelial Cell Rest of Malassez and Orthodontically Induced Inflammatory Root Resorption Repair

ECRM are clusters of epithelial cells derived from the HERS. These cells may persist throughout life and are usually found in the inner zone of the PDL close to the cementum.[299] The function of these cells are to protect against root resorption[300], participate in periodontal pocket and apical cyst formation[301-303] or maintain the PDL space and prevent dentoalveolar ankylosis.[72,304]

Reitan[7,305] has stated that ECRM were not present in periodontal tissue adjacent to areas of orthodontic root resorption and repair. This was because the epithelial rests atrophied during the initial hyalinisation induced by orthodontic forces and did not reappear during subsequent repair. Therefore, Reitan[7,305] concluded that epithelial rests could not possibly be involved in the restoration of the integrity of the ligament following orthodontic tooth movement. In addition, the significance of ECRM with regards to root resorption protection has been challenged in studies of PDL regeneration and apicoectomy repair.[247,306] A recent TEM study has indicated that these cells underwent decomposition and necrosis during the early phase of tooth movement in rats.[2] The proximity of blood vessels may be an essential factor in the resorption process and the epithelial rests may be implicated as a barrier to blood vessel access to the tooth surface. Once breached, the epithelial barrier may permit blood vessel access to the tooth surface and the resorptive process could begin and continue until the barrier is restored.

Brice *et al*[248] postulated that ECRM may be intimately involved in the repair of OIIRR and reconstitution of the periodontium following orthodontic tooth movement.

The study has revealed that epithelial cell clusters with ultrastructural features similar to ECRM were found in areas of repairing orthodontic root resorption after RME suggesting regeneration of epithelial cells may occur. The epithelial cell clusters appeared as dark staining cell clusters containing from two to eight cells, variably located between 10 and 100 μm from the repairing surface. The clusters were irregular in shape with a single nucleus towards the periphery and abundant dark cytoplasm. The cytoplasm contained numerous polyribosomes and free ribosomes but neither ER nor the Golgi apparatus were identified. This suggested that the cells synthesised protein as part of cytoplasmic renewal.[307] The feature of increased ribosomes, less prominent tonofilaments and infrequent desmosomes were very similar to the descriptions of proliferating human ECRM published by Yamasaki and Pinero.[308] Each cell within the epithelial cell cluster had one surface abutting the connective tissue. The irregular, pseudopodic, outer surface was encircled by a discontinuous basement membrane. The cytoplasmic processes projected out through the basement membrane into the adjacent connective tissue.

Wesselink and Beertsen[309] investigated the prevalence and distribution of ECRM after giving 1-hydroxyethylidene-1, 1-bispohosphonate (HEBP), a drug that interfered with the homeostasis in the PDL. Following treatment of HEBP, there was a severe reduction in width of the PDL at several sites resulting in ankylosis. After discontinuation of HEBP, the normal width of the PDL space was restored. Repair was characterised by root resorption, elimination of ankylosis, cementum formation and regeneration of ligament. These processes seemed to have no connection with the prevalence and distribution of ECRM. It was concluded that ECRM were unlikely to play an important part in the homeostasis of PDL and did not seem to be a prerequisite for its repair and maintenance.

Kittel and Sampson[310] evaluated the relationship between ECRM and RME induced root resorption craters in human. At the light microscope level of magnification, 1 μm toluidine blue section revealed epithelial cell clusters which variably stained either as dark cells against the tissue background or as pale staining cells. At the TEM level of magnification, the largest and most readily identifiable epithelial cell clusters were seen in the non-resorbed areas of the ligament. The least basophilic and hardest to identify epithelial cells were found within the resorption craters which could only be identified at the TEM level. The continuity of epithelial cells was demonstrated over short distances up to 38 μm in normal PDL and up to 18 μm in resorption craters. The epithelial cells were strands and branches instead of islands. This finding did not support the concept that epithelial cells act as a barrier to blood vessels but suggested that the blood vessels may have first migrated into the resorption crater following destruction of the epithelial network by PDL hyalinisation and inflammatory reactions to the orthodontic tooth movement.

Kittel and Sampson suggested several possible roles of ECRM in the initiation of cementum repair:

1. Release of collagenase inhibitor[311];
2. Production of an anti-invasion factor[71];
3. Promotion of cytodifferentiation of follicular cells to form new cementoblasts[312,313];
4. A physico-chemical barrier to cells and or products released from blood vessels once the epithelial cells became interposed between blood vessels and the cemental surface.

Hasegawa *et al*[314] studied the role of ECRM in PDL during cementum repair following experimental tooth movement in rats. ECRM were evident adjacent to

the resorption crater after seven days. At days 14 and 28, cementum repair was observed on the surface of the resorption crater. ECRM were not observed in the connective tissue of the resorption crater. Morphological studies showed that ECRM changed their distribution during cementum repair. ECRM adjacent to the site of root resorption were positive for ameloblastin during the early cementum repair phase. The ameloblastin might have induced mesenchymal cells differentiation into cementoblasts. The expression of Bone Morphogenic Protein-2 by ECRM suggested their role in signalling to surrounding mesenchymal cells and participating in cementum repair immediately after root resorption by epithelial mesenchymal interactions. ECRM were also positive for osteopontin during cementum repair. Osteopontin had many functions at various stages in the inflammation tissue repair cascade.[315] Osteopontin was a major extracellular matrix protein in calcified tissue and participated in cementum formation. It suggested that ECRM reproduced their potential to secrete and participate in cementum repair by the epithelial mesenchymal interactions.

6. Research Tools and Methodologies

Plain radiographs such as periapicals, DPT, lateral cephalograms are commonly used to detect root shortening. However, plain radiographs are 2D projection of a 3D object which has inherent magnification errors that make quantitative analysis questionable. In addition, root resorptions that lie parallel to the x-ray beam, e.g. on buccal and lingual surfaces of the tooth, cannot be detected. Therefore, plain radiographs are considered as a qualitative research tool in OIIRR.

Serial sectioning allows TEM examination and manual 3D reconstruction of the specimen. This type of 3D reconstruction using thin slices of 2D specimen could be cumbersome and not reliable as a quantitative analysis tool. This is because the shape, size and location of the resorption crater can be variable, therefore, resorption craters could be missed during sectioning. In addition, some specimen material may also be lost during sectioning which renders the accuracy in quantitative measurements.

SEM provides an overall image of resorption craters on the root surface with minimal tissue preparation. However, resorption craters are curved and absolute straight on view using SEM is difficult.[316] Parallax error in three dimensions could manifest as errors in measurements. This can be resolved by recording stereo images of resorption craters and converting into an 8-bit grayscale depth map.[14] This method has been shown to be highly accurate and reproducible.[316]

The use of the computed XMT system seems to be more superior in quantitative analysis of root resorption craters. Although time is required for specimen

scanning, there is no need for sample preparation or chemical fixation. Three dimensional reconstructions of the 2D images and volumetric measurements are performed by the associated computer software with high accuracy.

6.1 X-ray Micro-Tomography

6.1.1 History and Development

Wilhelm Conrad Roentgen reported the discovery of X-rays to the Wurzburg Physical-Medical Society on 28 December 1895.[317] The name X-ray was used by Roentgen due to the unknown nature of his discovery. X-rays allow non-invasive visualization of the internal structure of both the living and non-living objects. The limitation of conventional radiography is that it produces a 2D projection of a 3D object. Laminography or focal plane tomography can reproduce 3D structures from a 2D film.[318] This involves the translation of the object together with the detection medium in such a way that only one narrow slice parallel to the translation plane is in focus. The contrast from features outside the slice of interest is blurred which reduces its appearance from the image. The attainment of sharp images of the slices is challenging due to the thickness of each slice and the superimposed smearing of the images outside the region of interest.[318]

With the use of digital computers, laminography has evolved into a system that is used widely in the medical field i.e. computed axial tomography (CAT scan). In 1973, Hounsfield[319] was the first to develop a commercially available computed tomography system for medical imaging.

Computed tomography produces a 2D map of x-ray absorption in a slice of the subject. A series of x-ray projections are made through the slice at various angles around an axis perpendicular to the slice. Digital computing allows the series of projections or 2D absorption maps to be combined into the 3D map.[320]

Limitations in the use of this technology for patient application includes high radiation dosage for biological reasons and the long duration of the scan which results in involuntary movement by the subject and consequently image distortion.

There are four generations of computed tomography scanners. The first generation is known as the pencil beam system. It has the simplest arrangement consisting of the x-ray source, a pin-hole collimator and a single detector, as designed by Hounsfield.[319] The second generation scanner uses a parallel beam of x-rays and the third generation uses a flat fan distribution of x-rays. The fourth generation of computed tomography scanners uses a cone beam geometry of x-rays, which is a 3D analogue of the 2D fan beam geometry.[321] This design is ideal for volumetric computed tomography.

Advances in computed tomography have made possible the analysis of small specimen at high resolution with XMT. Conventional medical computed tomography scanners are able to achieve a resolution in the range of 1 to 2.5 mm. However, even with technological advances in detector design, it is unlikely the resolution can be improved due to X-ray dosage limitations.[320] On the other hand, XMT allows production of 3D images of small objects (2 to 50 mm). The resolution of XMT images is down to 1 μm but more typically to 5.0 to 10.0 μm which is approaching that of optical microscopy. In addition to the smaller scale,

unlike medical computed tomography scanners where the X-ray source moves around the subject, XMT rotates the specimen while the X-ray source and detector are stationary (Appendix 5).

Two-dimensional digital image dimensions are measured in units of pixels. A pixel is a 2D representation of the smallest unit of colour value within an image. This colour value may also be a shade of grey. Imaging software for computed tomography usually assigns the dimensions of a pixel to the x and y axis and it is able to represent a given area. The number of pixels per surface area may be used to describe the resolution of the image. Increasing the number of pixels per unit area improves image quality and resolution.[322] In 3D imaging, the unit voxel is used. A voxel is a 3D modification of a pixel with the added dimension of depth. Thus, a voxel has a volume and its dimensions have an x, y and z axis.[322]

6.1.2 The SkyScan 1172 Desktop X-ray Micro-tomograph

The SkyScan 1172 X-ray micro-tomograph (SkyScan, Aartselaar, Belgium) is a compact desktop system for microscopy and micro-tomography (Appendix 6). Its components include an X-ray shadow microscopic system and a computer with tomographic reconstruction software. Two-dimensional X-ray shadow projections of the sample are able to be reconstructed into a 3D representation of the sample including its inner structure. The SkyScan 1172 X-ray micro-tomograph is a fourth generation scanner. It has a cone beam X-ray source with a spatial resolution of 2 to 5 μm. The recommended image field width is 68 mm and a height of 70 mm. The sample is positioned on a rotating platform, which is programmed to revolve over either 180° if the object contains no highly radio-opaque structures or 360° if it

does. The distance of the platform from the X-ray source determines its magnification. Special filters can be used to reduce the noise in the reconstruction process. The X-ray detector consists of a high resolution charged coupled device with a resolution of 1024 x 1024 pixels. The images received are stored as 16 bit Tagged Image File Format (TIFF) picture files with a resolution of 1024 x 1024 pixels (Appendix 8). After image acquisition is completed, axial slice-by-slice reconstruction is performed by a software based on the Feldkamp cone-beam algorithm.[321] The resultant axial 2D images are generated as 1024 x 1024 pixel bitmap (BMP) images having an 8 bit gray-scale dynamic range (Appendix 9). The software package, VG StudioMax (version 1.2, Volume Graphics, GmbH, Heidelberg, Germany) is used to collate the axial 2D slices to form a 3D reconstruction of the images (Appendix 10).

6.1.3 X-ray Micro-tomography of Calcified Tissues

The applications of XMT systems are numerous and include fatigue analysis, performance prediction of materials, mineral analysis and the study of calcified tissues including bone and teeth.[323,324] Contemporary technology has mostly limited the use of XMT to inanimate and in vitro specimens. However, one in vivo study has been conducted to assess the changes in bone associated with ovariectomy in rats[325] and another in vivo study was performed on living snails.[326]

XMT have been readily used in recent years in research related to the field of dentistry. One study quantitatively determined the amount of mineral loss from human enamel rods after acid demineralization.[327] The deciduous incisors of low birth weight children were scanned by XMT to seek abnormalities in the mineral

concentration which may be related to developmental defects.[328] An XMT study of the mineral concentration of carious human dentine was of high enough resolution to detect calcified and enlarged tubular spaces in the lesion. The remineralised regions had a very high mineral content suggesting a loss of organic matter.[329] A study of rat lower incisors was performed with sequential XMT slices to measure differences in enamel mineral concentrations progressing from the apex to the incisal edge.[330] A 500 year old human tooth has also been studied with XMT, investigating features including attrition, cervical root caries, sclerotic dentine and calculus.[331]

XMT has been utilized to study the anatomy of root canals.[332] Some of the studies have involved extracted human teeth which were scanned before and after endodontic treatment to determine the area and volume changes related to instrumentation and obturation.[333,334] The resultant canal anatomy has also been compared following the use of different instrumentation techniques.[335,336]

There are a number of recent XMT studies involving bone. Comparisons have been made of biopsies from normal and synostosed human coronal sutures.[337] One study has involved finite element analyses of human trabecular bone architecture based on XMT images to obtain information about bone mechanical characteristics.[338] A comparison has been made of different bone graft approaches in rabbit mandibles. It was found that intramembranous bone grafts integrated better than endochondral bone grafts in three dimensions when they were grafted into membranous bony defects in the mandibles.[339] One group of authors have described the advantages of synchrotron radiation microtomography in quantifying the degree of mineralization of bone in three dimensions involving human iliac crest bone biopsy samples.[340] The process of tissue engineered bone growth into polymer scaffolds has been

studied with XMT technology at a resolution of 2 μm.[341] Bone structural changes caused by periapical pulpal infections in rats have been analysed.[342] A study involving rats during a postnatal acceleratory growth period was used to evaluate changes in the calcified tissue of the mandibular condyle from altered function. A maxillary occlusal splint was designed to shift the mandible laterally during closure. The authors found that compared to controls, the experimental animals' mandible and the condyle had a modified shape and size, including alterations in the trabecular bone of the condyle.[343] Cattaneo et al[344] investigated the stress and strain fields around orthodontically loaded dental implants using the finite element method and XMT and found individual differences in alveolar adaptation to orthodontic loading.

The micro-structure of bone around retrieved titanium micro-implants from humans has been investigated.[345] Another study to assess the osseointegration of a dental implant in a rabbit tibia has compared the quality of XMT, synchrotron radiation x-ray micro-imaging and conventional dental radiography.[346]

There are numerous studies in the orthodontic literature investigating root resorption using XMT technology. One study utilized X-ray microfocus tomographical scanning to assess a case of cervical external root resorption in human teeth.[347] A few studies have been conducted involving the assessment of OIIRR via XMT. A comparison was between zero, light and heavy orthodontic forces for the intrusion of human premolars.[16] It was found that the volume of resorption craters in the light and heavy force groups were two and four times greater than the zero force group respectively.[16] Another study involving rats was undertaken to investigate if fluoride had a beneficial effect in reducing the incidence of OIIRR. It was concluded that an average dose of fluoride reduced the size of resorption craters but

the effect was variable and found not to be statistically significant.[15] The use of XMT technology to volumetrically quantify OIIRR from clear removable thermoplastic orthodontic appliances has shown the amount of OIIRR was comparable to light orthodontic forces.[348] Ballard *et al*[349] have also quantified the volume of root resorption crater following intermittent orthodontic forces to be significantly lower than continuous orthodontic forces.