The Effect of Systemic Fluoride Intake on Root Resorption in Wistar Rats

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Declaration

CANDIDATE CERTIFICATE

This is to certify that the aforementioned candidate has carried out the work described in this thesis under the auspices of the Discipline of Orthodontics at The University of Sydney. This work has not been submitted to any other university or institution for a higher degree.

[Signature]

[Signature]
DEDICATION

This thesis is dedicated to my family and friends

who supported and encouraged me

through the passage of the last three years

with their love, advice and humour.
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"There are no principles; there are only events. There is no good and bad, there are only circumstances." — Honoré De Balzac
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1. INTRODUCTION

Root resorption is the process by which there is destruction of root structure due to physiological or pathological factors. Physiological resorption occurs during the exfoliation of the primary dentition and mesial drifting of the permanent dentition. Pathological resorption occurs subsequent to a traumatic injury, pathological disease process or iatrogenic causes.

The basis of orthodontic tooth movement is that if a sustained force is placed on a tooth, tooth movement will occur due to the remodelling of the bone around it. The bone remodelling process is mediated by the cells of the periodontal ligament. It has been suggested that heavy forces lead to the necrosis of cells in the periodontal ligament, causing hyalinization. Hyalinization is the sterile necrosis that occurs in localised pressure zones of the periodontal ligament. Until the hyalinized tissue is removed, tooth movement is delayed. During removal of the hyalinized tissue, the process of root resorption occurs. The hyalinized area evokes cellular reactions in the adjacent alveolar bone and root surface. Localised inflammatory processes recruit phagocytic cells such as macrophages, foreign body giant cells and osteoclasts to aid the removal of the hyalinized tissue.

Orthodontically induced inflammatory root resorption occurs commonly in patients undergoing orthodontic treatment. The incidence of root resorption can vary from 0% to
100% of treated patients. Lupi et al\textsuperscript{1111} reported that 73% of their orthodontically treated sample had radiographic evidence of root resorption. However, a smaller percentage (2%) showed severe resorption beyond one third of the original root length. The process of root resorption is very complicated and has multiple risk factors. With the advent of dental radiography, came the diagnosis of orthodontically induced root resorption, and thereafter the impetus for further investigations into the processes, cause and prevention of root resorption.

A recent study by Darendeliler et al\textsuperscript{2} investigated the physical properties and mineral content of cementum and its association with root resorption. The minerals studied were calcium, phosphate and fluoride. These minerals were studied because they are the primary constituents of the mineralized tissues of the cementum and an alteration in their quantity may alter the physical properties of cementum, thereby changing its susceptibility to root resorption. The trace element fluoride has long been recognised to have protective qualities when incorporated into mineralised tissues. Fluoride when taken up by hydroxyapatite, forms a more stable ionic structure, increases the crystal size and reduces its solubility. Studies have shown that fluoride is found in greatest concentration in cementum when compared to alveolar bone, dentine and enamel. The level of fluoride increases with age and also with the individual’s exposure to fluoride. However, due to the large inter-individual variability found in their collected samples, possibly due to the different fluoride exposure and age of their patients, no definitive conclusion could be derived as to its effect on the hardness of cementum.
When investigating the published literature on the possible protective qualities of fluoride against root resorption, most of the work has been done in the field of traumatic odontology\textsuperscript{3-10}. It has been shown that teeth that have been avulsed, when treated topically with a fluoride varnish, have a reduced incidence of root resorption. Fluoride when applied to injured periodontal tissue in rats, has also shown promising protective qualities. However, there have not been any studies investigating the effect of systemic fluoride intake on orthodontically induced root resorption.

The aim of the present study is to investigate if fluoride has a protective effect on the cementum surface against orthodontically-induced inflammatory root resorption. Root resorption at this stage is still largely unpredictable, considering the multi-factorial nature of the process. If an increased fluoride intake can improve the cementum surface physical property against the unwanted effects of orthodontically-induced root resorption, then this may present itself as a useful clinical therapeutic agent.
2. LITERATURE REVIEW

2.1. Root Resorption

2.1.1. Introduction

External root resorption is a common occurrence during orthodontic treatment, which involves the removal of cementum and/or dentine, usually resulting in the shortening of the root structure. Radiographic evidence of root resorption was presented by Ketcham\textsuperscript{11,12} and since then, it has been recognised as an undesirable side effect of orthodontic treatment.

2.1.2. Definition

Brudvick and Rygh\textsuperscript{13} described root resorption as the removal of mineralised cementum and dentine associated with the remodelling process of the periodontal ligament, more specifically, during the removal of hyalinized necrotic tissue.

2.1.3. Classification

Andreasen\textsuperscript{14} defined three types of external root resorption:

1. Surface resorption, which is a self limiting process, involving small surface areas followed by spontaneous repair from the adjacent periodontal ligament.
2. Inflammatory resorption, where the initial root resorptive process has reached the dentinal structures of the infected necrotic pulpal tissues or an infected leukocytic zone.

3. Replacement resorption, where bone replaces the resorbed root surface, eventually leading to ankylosis.

Tronstad 15 defined two types of external root resorption

1. Inflammatory resorption, which occurs when the predentin or precementum becomes mineralised, or in the case of precementum, is mechanically damaged or scrapped off. There are two types of inflammatory resorption described:

   i. Transient inflammatory resorption, where root damage is minimal and the stimulation for the resorptive process is of a short duration. This form of resorption is usually undetectable radiographically and is repaired by a cementum-like tissue.

   ii. Progressive inflammatory resorption, where the resorptive stimulus extends for a longer duration, leading to a radiographically observable change in root structure.

2. Replacement resorption is where bone is deposited in sites of tooth resorption.
Ghafari\textsuperscript{16} has classified four types of external root resorption:

1. Inflammatory resorption, where resorption is a result of an inflammatory response to a specific stimulus. Inflammatory mediators and phagocytic cells colonise the mineralised or denuded cemental surface and later dentinal tubules or pulpal tissues.

2. Surface resorption occurs in the outlying area of the root surface (cementum, possibly dentine and pulpal tissue). This is a transient inflammatory process which is followed by repair with a cementum-like tissue. This is generally undetected radiographically.

3. Progressive resorption with bone substitution, where the inflammatory resorptive process has resulted in bone formation within the space of the resorbed tooth structure. This resorption is detectable radiographically, and ankylosis may be the final outcome.

4. Progressive resorption without bone substitution, where there is a progressive resorptive process without the substitution of bone within the resorption area. This kind of resorption may be cervical or peripheral, but has not been reported as a common post-orthodontic complication.

2.1.4. Prevalence

Root resorption has been reported in populations that are orthodontically treated and untreated. During orthodontic tooth movement root resorption is likely to occur, causing
root shortening. However, most resorption is of a minor nature, causing minimal changes to the root length\textsuperscript{17,18}. There is a high risk group where severe resorption may occur which comprises one to three percent of the population\textsuperscript{19-21}.

2.1.5. Aetiology

Orthodontic root resorption appears to be of multi-factorial in aetiology. The factors implicated can be divided into patient-related and treatment-related factors\textsuperscript{22}.

2.1.5.1. Patient related factors

2.1.5.1.1. Individual susceptibility

The incidence of root resorption varies amongst persons and within the same person at different times\textsuperscript{23}. Root resorption also occurs in varying degrees in different teeth\textsuperscript{24,25}. Becks\textsuperscript{26,27} stated that orthodontic root resorption was the result of an individual’s predisposition due to endogenous factors, and recent findings are beginning to unravel this association. Harris et al\textsuperscript{28}, studied the genetic contribution to external apical root resorption within and amongst siblings and found a hereditary component. Al Qawasmi et al\textsuperscript{29,30} analysed the effect of the interleukin IL-1 genes and external apical root resorption, finding that their samples homozygous for the IL-1B allele 1 had a 5.6 fold risk of external apical root resorption of greater than 2mm, as compared to those who were not homozygous.
2.1.5.1.2. Systemic factors

2.1.5.1.2.1. Asthma/Allergy

McNab et al\textsuperscript{31} showed a higher incidence of apical root resorption in asthmatics, irrespective of medication, when compared to non-asthmatics. The increased incidence of external apical root resorption was confined to an increase in root blunting (grade 1). The authors suggested that the inflammatory mediators associated with asthma may enhance root resorption.

Davidovitch et al\textsuperscript{32} looked at a sample of 102 orthodontically treated patients who experienced root resorption in one or more teeth and compared them with a control sample of 102 orthodontically treated patients who had no root resorption. He found an increased incidence of root resorption in patients who had asthma and allergies.

Owman-Moll and Kuro\textsuperscript{33} studied a sample of 50 individuals that had orthodontic tooth movement of their maxillary premolars, to define risk factors associated with root resorption. They found that there was an association between allergies and the extent of root resorption, although this was not statistically significant.

2.1.5.1.2.2. Alcohol consumption

Ethanol inhibits the hydroxylation of vitamin D3 in the liver, thereby impeding calcium homeostasis and causing a rise in parathyroid hormone. This has the
additional effect of enhancing the resorption of mineralised tissues, including cementum, leading to increased root resorption\textsuperscript{34}.

\textbf{2.1.5.1.2.3. Endocrine Imbalance}

Endocrine problems relating to bone metabolism have been related to root resorption. The metabolism of mineralised tissues in the body is primarily controlled by three hormones\textsuperscript{35}. 1,25-Dihydroxycholecalciferol is a steroid hormone formed from vitamin D by successive hydroxylation in the liver and kidneys. Its primary function is to increase calcium absorption in the kidney. Parathyroid hormone is secreted by the parathyroid glands and its main action is to mobilise calcium from bone and increase urinary phosphate excretion. Calcitonin is secreted by the thyroid gland and inhibits bone resorption. All three hormones operate in concert to maintain the constancy of the calcium level in body fluids. A fourth hormone, parathyroid hormone related protein (PTHrP), acts on the parathyroid hormone (PTH) receptors and is important in skeletal development in utero. Other hormones that may play a role in calcium metabolism are phosphate-regulating hormone, glucocorticoids, growth hormone, oestrogens and various growth factors.

Therefore, it is not unusual that altered endocrine conditions such as hyperparathyroidism\textsuperscript{36,37}, Paget's disease\textsuperscript{38}, hypophosphataemia\textsuperscript{39}, hypothyroidism, hypopituitarism and hyperpituitarism\textsuperscript{27,40} are associated with altered resistance to root resorption.
Goulschin et al.\textsuperscript{36} did a review article on root resorption and presented a patient with hyperparathyroidism who had external and internal root resorption in most of the teeth examined.

Paget’s disease of bone is a chronic skeletal disorder which may result in enlarged or deformed bones in one or more regions of the skeleton. This disease causes an increased and irregular formation of bone as the bone cells which are responsible for bone remodelling are out of control. The cause of this metabolic disease is not known. Smith\textsuperscript{38} presented a case report of a patient who had Monostotic Paget’s disease of the mandible with progressive resorption of teeth over a period of 7 years. Histological specimens were examined and multi-nucleated cells (odontoclasts) were seen at the resorbing surface of dentine, which in some areas were being replaced by acellular coarse-fibered bone. Hypercementosis of the roots of teeth in Paget’s disease of the jaws is a common finding. However, Smith indicated that root resorption may precede hypercementosis and even accompany it to some extent. Rushton\textsuperscript{41} stated that with the early osteolytic phase of the disease, where bone resorption predominates, the teeth exhibit a resistance to the resorptive process. When root resorption occurs, additional factors, “either mechanical or toxic”, were present, although he did not specify the exact nature. The pathologic resorption of dentine and cementum proceeds by a similar mechanism to that causing bone resorption.
2.1.5.1.2.4. Nutrition

Engstrom et al\textsuperscript{37} demonstrated root resorption in animals deprived of dietary calcium and vitamin D. Male albino Sprague Dawley rats were divided into 2 groups. One group was fed a normal diet. The other group was fed a low calcium diet deficient with vitamin D. After 3 weeks on this diet, both groups had an expansion appliance placed around their maxillary incisors. After 14 days, 40 rats from both groups were euthanised. The remaining 40 rats from both groups were killed 7 days later. Their results showed that the diet deficient in calcium and vitamin D induced hypocalcaemia and an increased alkaline phosphatase activity. The serum content of circulating parathyroid hormone was also higher in the diet deficient group, as compared to the controls (secondary hyperparathyroidism). After 7 days, tooth movement was found to be greater in the diet deficient group. They reported more rapid and extensive bone resorption in the compression zone of the diet deficient group. They concluded the severity of root resorption was increased after orthodontic treatment and was related to an increase in alveolar bone resorption. In short term experiments, parathyroid hormone has been shown to increase the number of osteoclasts present in periodontal ligament\textsuperscript{42}.

Midgett et al\textsuperscript{42} studied the effects of nutrition induced hyperparathyroidism on bone remodelling during orthodontic tooth movement in beagle dogs. The experimental group of beagles received a diet deficient in calcium and phosphorus. They found that the reduction in calcium and phosphorus induced an osteoporotic condition.
Histological sections of the dogs' mandibles revealed a large loss of trabecular bone, while cortical bone remained unchanged. Examination of the parathyroid glands indicated that the dogs were in a hyper-parathyroid state which accounted for the trabecular bone loss.

However, Linge and Linge\textsuperscript{21} and Goldie and King\textsuperscript{43} suggested that nutritional imbalance is not a major factor in root resorption during orthodontic treatment. Goldie and King\textsuperscript{43} studied root resorption and tooth movement in orthodontically treated, calcium deficient and lactating Sprague Dawley rats. Their samples were divided into 2 groups; control group (n=10) were non-pregnant and non-lactating; test group (n=25) which were lactating and kept with their young. Only the test group was fed a diet with reduced calcium and phosphorus, while the control group was fed a normal laboratory diet. A closed coil spring was activated and connected to the maxillary first molar and incisors to produce tooth movement. Their results showed: the magnitude of tooth movement was greater in the test group; the test group had significant loss of bone mass; and root resorption craters were, in general, less in the test group than the controls.
2.1.5.1.3. Age

2.1.5.1.3.1. Chronological

With increasing age there are changes to hard and soft tissues, including; reduced vascularity, plasticity and width of the periodontal ligament, increased density and reduced vascularity of bone and an increase in the width of cementum. Reitan\(^{44}\) suggested that these changes are responsible for the increased incidence of root resorption seen in adults. Most studies agree that adults patients are more susceptible to root resorption\(^ {20,21,44-50}\), but a few researchers disagree\(^ {24,25}\).

2.1.5.1.3.2. Dental

Partially formed roots appear to develop normally during orthodontic treatment and it has been suggested that teeth with open apices may be more resistant to apical root resorption\(^ {21,51,52}\). Hendrix et al\(^ {53}\) however, found that teeth with incomplete root formation during orthodontic treatment “did not reach normal tooth length”. This study however, employed panoramic radiographs and calculated tooth length for maxillary and mandibular teeth, without adjustments for radiographic projection differences in the pre- and post- treatment films\(^ {22}\).
2.1.5.1.4. Habits

Several habits have been reported to increase the risk of external apical root resorption. Large, non-physiological forces may contribute to root resorption and mechanisms are probably similar to orthodontic tooth movement. Nail biting, finger sucking beyond 7 years of age and lip or tongue dysfunction have been shown as risk factors to root resorption.

2.1.5.1.5. History of trauma

A history of trauma may increase the risk of root resorption during orthodontic treatment. Linge and Linge found that traumatised teeth on average lost 1.07 mm of root structure compared to 0.64 mm in non-traumatised teeth. Traumatised teeth that are not inflamed and without previous resorption, may not be more susceptible to resorption than non-traumatised teeth. However, it is thought that the initiation of orthodontic force on a traumatised tooth may aggravate the resorptive process.

2.1.5.1.6. Ethnicity

Sameshima and Sinclair studied a sample of 868 patients and found that Asian patients experienced less root resorption compared to white or Hispanic patients. However, Sameshima and Sinclair’s more recent article suggested that their sample
population was disproportionate when comparing the Asian and African American samples to the larger Caucasian sample.

2.1.5.1.7. Endodontically treated teeth

Mirabella and Artun\textsuperscript{18} and Thilander et al\textsuperscript{49} found endodontically treated teeth had less resorption. Remington et al\textsuperscript{59} suggests that an increased density of dentine in root filled teeth provides resistance to resorption. However, patients with an active inflammatory process from infected pulpal tissue, or from trauma, may be more susceptible to root resorption as orthodontic tooth movement itself creates an inflammatory response that may increase the resorptive process. A successful endodontically treated tooth, with healthy surrounding tissues, in the absence of inflammation, would be no more susceptible to resorption than a normal tooth.

2.1.5.1.8. Malocclusion

Linge and Linge\textsuperscript{55}, Harris and Butler\textsuperscript{60}, Hendrix et al\textsuperscript{53} and Sameshima and Sinclair\textsuperscript{50} have found that an increased overjet is a risk factor for root resorption. Harris and Butler\textsuperscript{60} also linked the presence of an openbite with root resorption.
2.1.5.2. Treatment related factors

2.1.5.2.1. Duration

Most studies agree that as the duration of orthodontic treatment increases, the risk and severity of external apical root resorption increases as well\textsuperscript{22,45,47,48,51,55,61-74}. Sameshima and Sinclair\textsuperscript{74} looked at a sample of 868 patients collected from 6 different specialist practitioners and found longer treatment time to be significantly associated with increased root resorption for maxillary central incisors. However, the authors did question the reasons for the extended period of treatment, be it due to an uncooperative patient, longer finishing and detailing period or a heroic non-surgical plan. The exact reasons were not investigated, but may shed light into the exact reasons for the increase in root resorption seen in these patients.

However, other studies have not supported this finding\textsuperscript{46,75}.

2.1.5.2.2. Appliance type

Fixed appliances have been shown to cause more root resorption than removable appliances\textsuperscript{55}. This is probably due to the range of tooth movement available via fixed appliances. Mavragani et al\textsuperscript{76} demonstrated that the standard edgewise technique produced more root resorption than the straight wire technique. No difference was found between the Speed appliance and Edgewise techniques\textsuperscript{77} and between sectional and continuous mechanics\textsuperscript{78}. A recent study\textsuperscript{79}, however, found an increased root
resorption in patients treated with the Speed appliance when compared to TipEdge and MBT appliances. Kinsella\textsuperscript{80} found less resorption in patients treated with the Begg appliance as compared to the Edgewise appliance. Malmgren et al\textsuperscript{81}, however, found no differences. Linge and Linge\textsuperscript{21} suggested the use of inter maxillary elastics increased the amount of root resorption but Sameshima and Sinclair\textsuperscript{74} did not find any correlation.

The use of rapid maxillary expanders has been shown to cause root resorption\textsuperscript{49,70,82,83} but Sameshima and Sinclair\textsuperscript{58} did not find any correlation between the amount of root resorption and expansion. However, none of the severe cases studied by Sameshima and Sinclair had expansion, while only two of the controls had a rapid palatal expander.

2.1.5.2.3. Nature of applied force

Schwartz\textsuperscript{84} proposed that orthodontic movement greater than the partial pressure of the periodontal capillaries (26gm/cm\textsuperscript{2}) causes periodontal ischaemia that may lead to root resorption. Since then various animal studies\textsuperscript{70,85,86} and human studies\textsuperscript{2,65,87,88} agree that there is an increase in severity of root resorption with increasing force magnitude.
King and Fischlschweiger\textsuperscript{86} applied various forces (50g to 200g) in rats and found that lighter forces produced more rapid tooth movements, whereas moderate to heavy forces produced slower tooth movement. They also demonstrated that light forces produced less resorption to the cementum.

Harry and Sims\textsuperscript{65} examined extracted human premolar teeth that had 50g, 100g and 200g of intrusive force, with a scanning electron microscope. They concluded that higher forces increased the stress to the root surface and increased the rate of lacunae development, thereby increasing root resorption.

Faltin et al\textsuperscript{87,88} also examined human premolar teeth which had undergone intrusive forces of 50\text{cN} or 100\text{cN}. They found greater root resorption in the teeth that had undergone greater intrusive force.

More recently, volumetric analysis of resorption craters was examined in extracted human teeth, comparing controls with a force of 25g or 225g, with buccal displacement\textsuperscript{89} or intrusion\textsuperscript{90}. These studies have also agreed with previous studies showing higher forces increased the amount of external root resorption.
However, other studies have contraindicated these findings. Reitan\textsuperscript{47} examined 72 premolars after application of 25g to 240g of intrusive, extrusive and tipping movement over a period of 10 to 47 days and found that external root resorption was poorly correlated to force magnitude.

A series of studies by Owman Moll et al\textsuperscript{91,92} looked at tooth movement with regard to different force applications (50g vs. 100g, 1995; 50g vs. 200g, 1996). They found no significant differences in the frequency and severity of root resorption, but there were large inter-individual variances. They concluded that root resorption was independent of force magnitude, but that individual reactions may be more important.

\textbf{2.1.5.2.4. Types of tooth movement}

Teeth undergoing orthodontic treatment can be displaced by tipping, torque, intrusion, extrusion and bodily movements. However, it seems that intrusion is the most likely to cause root resorption\textsuperscript{47,62,93}. Reitan\textsuperscript{44,94} and Thilander et al\textsuperscript{49} suggested that there is less root resorption associated with bodily movement compared with tipping due to the stress distribution along the roots.

There are conflicting reports as to whether continuous or intermittent forces produce more or less root resorption. A pause in tooth movement allows the resorbed
cementum to heal and many believe that discontinuous forces produce less root resorption\textsuperscript{45,94-100}.

Acar et al\textsuperscript{99} examined 22 human teeth from patients 15 to 23 years of age, after application of a continuous 100g tipping force on one side and an intermittent (12 hours per day) force on the other side, over a period of 9 weeks. The force applied was through the use of elastics. Their results showed that the intermittent forces resulted in less root resorption. A critical analysis of the study by Dr King, questioned the compliance of the subjects studied. Also maxillary and mandibular premolars were treated as equivalent teeth, even though there is evidence that the different teeth respond differently to root resorption.

Weiland\textsuperscript{100} studied 84 premolars from patients, aged between 10.2 to 14.5 years, which had been moved buccally with an orthodontic appliance. The experiment was a split mouth design, where the premolar on one side was activated with a stainless steel wire (0.016 inch) and the contralateral premolar was moved with a super-elastic wire (0.016 inch). The stainless steel wire was reactivated every four weeks and the experiment concluded after 12 weeks. Their results showed that the teeth activated with the super-elastic wire moved significantly more, but had greater perimeter, area and volume of resorption (140% more) than the teeth with stainless steel wire. However, the depth of the resorption lacunae did not differ significantly.
Owman Moll et al.\textsuperscript{92} examined 32 maxillary first premolars in patients aged between 11.8 to 15.8 years, after application of a buccally directed force of 50g. They found no difference in the amount or severity of root resorption between forces applied continuously or intermittently.

2.1.5.2.5. Amount of tooth movement

Sameshima and Sinclair\textsuperscript{74} surmised that root resorption is directly related to the distance moved by the tooth roots. This has been alluded to by previous authors\textsuperscript{46,67,74,93,101}. The maxillary incisors tend to be moved more than any other teeth, and accordingly is one of the teeth most at risk of root resorption. Sameshima and Sinclair\textsuperscript{58} found that severe root resorption occurred in their samples when the root apex was displaced lingually, a mean difference of 1mm more than the control group. Extractions were not found to be a significant factor in the severity of root resorption in maxillary incisors, as extractions for severe crowding do not impact upon the movement of the maxillary incisors as much as the displacement for overjet correction.
2.1.6. Mechanism of Root Resorption

Inflammation is a local response to injury, commonly as a reaction to microbial infiltration, but also to chemical and physical stimuli. The inflammatory process is characterised by five cardinal signs of redness, heat, swelling, pain and loss of function. The inflammatory response eliminates the pathogenic insult and removes the injured tissue components. This process accomplishes regeneration of the normal tissue arrangement and return of physiological function. The mechanisms responsible for the localisation and removal of injured tissues are initiated by the recognition that tissue injury has occurred. This is followed by an amplification stage of the inflammatory response, in which both soluble mediators and cellular inflammatory systems are activated.

The associated tissue changes include

a) localised vasodilation with increased vessel permeability and blood flow
b) exudation of plasma fluids
c) leukocytic migration into extra vascular spaces.

There are also a number of endogenous chemical mediators, such as histamine, prostaglandins, leukotrienes and cytokines that can mediate blood flow, augment adherence to vascular endothelium and promote migration of leukocytes into tissues.
Tooth movement occurs when there is a prolonged application of force that exceeds the bio-elastic limits of tooth supporting structures\textsuperscript{102}. These forces induce a physical stimuli which start an inflammatory reaction in the connective tissues, leading to adaptive remodelling of the periodontal ligament and alveolar bone\textsuperscript{102-104}. Over compression of the periodontal ligaments leads to a localised ischaemia and cell death, forming an area of necrotic or hyalinised tissue\textsuperscript{103,105-109}. These tissue changes are sterile and promote an inflammatory reaction in an attempt to remove the hyalinised tissues and initiate tissue repair\textsuperscript{103,110-112}.

Research has shown that external root resorption during orthodontic tooth movement is associated with the localised over compression of the periodontal ligament, the formation of the hyalinised tissue/zone and its subsequent removal. The exact mechanism of root resorption is yet to be fully explained by the available literature, but the primary process is of an inflammatory reaction to the formation of hyalinized tissue\textsuperscript{23,105,113,114}. Studies and investigations into the histological changes in the surrounding tissues have mainly been done in animal samples particularly rats.

Initially it was believed that an osteoclast-like cell was the main initiator of root resorption\textsuperscript{23,66,105,107,115}. During the process of bone remodelling, the surrounding osteoclasts show a high content in tartrate-resistant acid phosphatase (TRAP)\textsuperscript{116,117} and it
is this content that can be stained positive for those cells involved in orthodontic tooth movement and root resorption\textsuperscript{117-119}.

Brudvik and Rygh studied the processes of root resorption and repair and published their results in a series of papers. Brudvik and Rygh\textsuperscript{120} studied the histological changes seen under light microscopy in rats (21) and mice (31) molar teeth that had undergone orthodontic tooth movement. The stains used in this study were Tartrate Resistant Acid Phosphatase (TRAP) and Haematoxylin & Eosin (H&E). They concluded that the reactions of and penetration by adjacent periodontal membrane cells into unmineralized pre cementum is the first phase leading to root resorption. The first resorptive attack on the root surface occurred in the area of the over-compressed zone. The surrounding cells invading and removing the hyalinized tissue are from adjacent healthy periodontal membrane where they are stationary or blood borne. Root resorption occurred in the circumference of the hyalinized tissue after 2 to 3 days in their rat samples. Prior to this, mononucleated cells were observed close to or impinging onto the root surface. These cells are postulated to start the resorption of the unmineralized cementoid. They suggested that some of these cells were macrophages, fibroblasts or cementoblasts, which were all TRAP-negative. Brudvik and Rygh\textsuperscript{121} using transmission electron microscopy, studied in greater detail the penetration of cells into pre cementum and mineralized cementum. They summarized that mononucleated non-clast cells were found during the initial removal of pre cementum and mineralized acellular cementum at the periphery of the hyalinized tissue and also some distance from it. Macrophage-like cells
phagocytosed necrotic tissue in the periodontal ligament after 6 hours and near the root surface after 24 hours. Fibroblast-like and cementoblast-like cells were involved in the resorptive process of precementum after 24 hours. The surface layers of mineralized cementum were removed by mononucleated cells after 3 days. Multi-nucleated cells were seen in surrounding tissues but not usually near the mineralized cementum surface during their 5 day test period.

Brudvik and Rygh\textsuperscript{13,122} (1994) investigated further into the resorptive processes beneath the main hyalinized zone. They found that the majority of cells involved in the removal of the hyalinized tissues and root resorption were multinucleated and TRAP positive. The root surface adjacent to the hyalinized tissues underwent degenerative changes. They found that the cells involved in the removal of the main part of the necrotic periodontal membrane, as well as the root surface tissue, differ from those involved during the initial phase. Using transmission electron microscopy, Brudvik and Rugh\textsuperscript{13} examined the cells involved in the removal of the main hyalinized tissue and those involved in root resorption. Twelve Wistar rats were studied and the results indicated that multi-nucleated giant cells with non-ruffled borders were involved in the removal of necrotic tissues and root resorption. Mono-nucleated giant cells with ruffled borders were never observed near the remnants of necrotic tissue, but were found in the resorption lacunae of root and bone surfaces. Cementum fragments were not found inside these cells and they postulated that removal of cementum tissue occurred extra cellularly. They also suggested that the multi-nucleated cells are derived from monocytes
and macrophages that initially invaded the necrotic hyalinized tissues. These observations have since been supported by recent publications\textsuperscript{112,123}.

2.2. Cementum

2.2.1. Definition

Cementum is the mineralized dental tissue covering the anatomic roots of teeth. It begins at the cervical portion of the tooth at the cemento-enamel junction and continues to the apex. Cementum is the surface for attachment of collagen fibres that bind the tooth to surrounding structures. It is a specialized connective tissue that shares some physical, chemical and structural characteristics with compact bone\textsuperscript{124}.

Cementum is different from bone in that it is not innervated, exhibits little or no remodelling and is avascular. Despite these differences cementum shares some characteristics with bone.

Firstly, diseases that affect the properties of bone often alter cementum’s properties as well. Paget’s disease results in hypercementosis, hypophosphatasia results in a lack of cementum formation with exfoliation of teeth, and decreased cementum is associated with hypopituitarism.
Secondly, the composition of cementum is similar to bone (see below).

2.2.2. Chemical composition

In fully matured teeth, (dry weight) cementum contains about 45% to 50% inorganic material and 50% to 55% organic material and water\textsuperscript{124}. The inorganic material consists of calcium and phosphate in the form of hydroxyapatite. Numerous trace elements are found in cementum in varying amounts. Fluoride found in cementum is by far the most concentrated of all mineralized tissues\textsuperscript{125,126}.

2.2.3. Distribution

Cementum varies in thickness at different levels of the root. Cementum is thinnest at the cementoenamel junction (20 to 50 μm) and thickest towards the apex (150 to 200 μm)\textsuperscript{124}, although it may exceed 600μm\textsuperscript{127}. Cementum continues to increase in thickness with age. This observation has been noted by a number of authors in the past from G.V. Black\textsuperscript{128} to Weinmann and Sicher\textsuperscript{129}. Indeed, this was demonstrated by Zander and Hurzeler\textsuperscript{130} who studied 233 single rooted teeth extracted from people ranging in age from 11 to 76 years. They found that the cementum thickness tripled in the group aged 51 to 76 years (0.215 mm) as compared to those less than 20 years (0.076 mm) of age.
2.2.4. Classification

With light microscope, two kinds of cementum can be seen, acellular and cellular\textsuperscript{124,127}. Acellular cementum describes the layers of cementum that do not incorporate cells. Acellular cementum covers the root dentine from the cementoenamel junction to the apex, but it is often missing in the apical third of the root. Cellular cementum contains cementocytes in their lacunae, and is located mainly at the apical third portion of the tooth root in humans.

Cementum can further be classified according to the time of formation (primary or secondary), the presence or absence of cells within its matrix (acellular or cellular) and the origins of collagenous fibres of the matrix (intrinsic or extrinsic)\textsuperscript{131}.

a. Acellular A fibrillar Cementum
b. Primary Acellular Extrinsic Fibre Cementum
c. Primary Acellular Intrinsic Fibre Cementum
d. Secondary Cellular Intrinsic Fibre Cementum
e. Secondary Cellular Mixed Fibre Cementum
f. Cellular Mixed Stratified Cementum
g. Intermediate Cementum
2.3. Fluoride

Fluorine (symbol of F) has an atomic weight of 19, is a chemical gaseous element of the halogen group. It is the most electronegative of the elements of the periodic table. It is the 13th most abundant element on the earth’s crust. As such, it has been found in a wide range of concentrations in virtually all inanimate and living things. Owing to its extreme activity, it is one of the most chemically active substances known. It is found in both organic and inorganic compounds.

Fluoride enters the atmosphere by volcanic action. This is then trapped by soil and water due to the action of wind on these surfaces and the deposition of dust, rain, snow or fog. It enters nature’s water supplies by leaching from soils and minerals. Fluoride enters vegetation by uptake from soil and water and by absorption of fluorides from the air.

2.3.1. History of Fluoride

Fluoride is the ionic form of fluorine. Fluoride content in teeth was first investigated by Ehrhardt, a German scientist, in 1847. Later in 1892, Sir James Crichton Browne, in an address to the British Dental Association, stressed the importance of a fluoride supply during the development of teeth and the consequence of its deficiency in the production of inferior enamel. Around the 1930's it was demonstrated that fluoride in small dosages have a remarkable protective effect against caries. An American chemist, H.V.
Churchill, found fluoride of some 14ppm in the water supply of a community with extreme mottling. The result of this research led to confirmatory observations from all over the world associating high concentrations of fluoride with enamel mottling. Observers also became aware that the incidence of caries was reduced (50-60%) in the communities where mottled enamel occurred\textsuperscript{132}. Further research into the biology of fluorides has since been extensive. The discovery of the connection between excessive content of fluoride in water and enamel mottling, was the starting point of research into fluorides and hard tissue physiology and pathology.

Fluoride has been shown to reduce the number of carious lesions in low doses, but in higher doses, can cause disturbances in enamel formation. Fluorine is the most exclusive bone seeking element, due to its great affinity for calcium phosphate. It is, therefore, accumulated in every tissue that undergoes calcification, be it physiological or pathological.

In bones, fluorine increases the size of the apatite crystals and reduces their solubility. This has naturally led to expectations of a positive role of fluorine as a skeletal stabiliser. Large doses of sodium fluoride have been tried as a therapeutic agent in the treatment of osteoporosis and Paget’s disease, but not always with consistent success.
2.3.2. Fluoride metabolism

Fluoride is absorbed by the body by way of the lungs and/or gastrointestinal tract. Fluoride is transported within the body via the plasma. Within minutes after ingestion, increased plasma fluoride levels can be detected in plasma\textsuperscript{133}. The peak usually occurs during the first hour after ingestion. The plasma levels then show a rapid decline due to the uptake by calcified tissues and urinary excretion. About 50\% of the absorbed fluoride will be excreted in the urine during the following 24 hours while most of the remainder will become associated with calcified tissue. Approximately 99\% of fluoride in the body is associated with calcified tissues\textsuperscript{134}. However, the fluoride in calcified tissue is not irreversibly bound and can be released.

2.3.3. Fluoride intake

The fluoride intake of most humans is chiefly derived from dietary sources. However, recent studies also show that excessive amounts of fluoride can occur through the inadvertent swallowing of fluoride-containing dentifrices\textsuperscript{135}.

Fresh water fluoride levels vary from less than 0.1 to over 10 ppm depending on the amounts and solubilities of the compounds available in the local soil. Fresh or unprepared foods generally have a fluoride concentration from between 0.01 to 1 ppm\textsuperscript{133}. The major exception to this is marine fish and tea. The oceans have a fluoride concentration of approximately 1.5 ppm which continuously exposes marine plants and animals to large amount of fluoride. The fluoride concentrations of dry tea vary (4 – 400
ppm) while those of brewed tea range from 1 – 6 ppm depending on the amount of tea used, water fluoride content and brewing time\textsuperscript{136}.

2.3.4. Fluoride Absorption

2.3.4.1. Oral Absorption

When taken by mouth, the absorption of fluoride begins in the oral cavity. Gabler placed neutral solutions containing fluoride into the mouths of rats. He concluded that absorption occurred at a measurable but substantially lower rate\textsuperscript{137}. Patten determined that only about 7\% of the applied dose was absorbed over a 2.5 hour period\textsuperscript{138,139}. Similar experiments also showed that the pH and concentrations of fluoride tested had no influence on its permeability through epithelium\textsuperscript{139}.

2.3.4.2. Gastrointestinal Absorption

Fluoride absorption in the gastrointestinal tract is rapid and essentially complete. Studies have shown that high levels of ions such as calcium, aluminium and magnesium can react with fluoride and reduce its solubility\textsuperscript{136}. Sodium fluoride in water is essentially completely absorbed. If fluoride is taken with milk or with food, the degree of absorption is reduced because insoluble complexes are formed\textsuperscript{140}. The ability of calcium to reduce the absorption of fluoride is the basis for treating acute fluoride toxicity with gastric lavages of calcium containing solutions.
2.3.5. Plasma Fluoride

In general, there are two forms of fluoride in human plasma\textsuperscript{141-143}. One form is the ionic form (or inorganic or free fluoride). This is the form of fluoride that is of significance to dentistry, medicine and public health. It is detectable with an ion-specific fluoride electrode. The other form is the non-ionic or bound fluoride. It appears to be composed of lipid soluble organic fluorocompounds (which are not detectable by the electrode). The biological significance of this form has not been determined. The ionic and non-ionic forms constitute the total plasma fluoride.

Unless the intake of ionic fluoride is unusually high, the non-ionic form of fluoride is found in higher concentration\textsuperscript{142,143}. The ionic form of fluoride will be comparably higher in human samples with a drinking water fluoride level of 5.6 ppm\textsuperscript{133}. The non-ionic fluoride in plasma does not increase with increasing ionic fluoride intake\textsuperscript{142}, but ionic fluoride does\textsuperscript{142,144}.

Plasma fluoride levels in rats tend to be lower than in humans\textsuperscript{145}. Turner et al\textsuperscript{146}, looked at the effect of different levels of fluoridated water and bone strength. They suggested that the plasma fluoride levels of their Sprague Dawley rats, given 5, 15 and 50 mg/L fluoridated water (5, 15 and 50 ppm) would be equivalent to those measured in humans consuming fluoridated water of 1, 3 and 10 mg/L respectively (1, 3 and 10 ppm). The use of fluoridated water to a concentration of 100 ppm in rats would, therefore, be equivalent to 20mg/L or 20 ppm in humans.
2.3.6. Toxicity

Fluoride is also considered a toxic substance. Acute ingestion of large quantities may be followed by rapidly developing signs and symptoms, which may eventually result in death. When ingested in small amounts during the period of tooth development, it may produce changes in the quality and appearance of enamel (i.e. dental fluorosis)\textsuperscript{147}. When larger amounts are ingested, over a period of years, the quality and quantity of skeletal structures are altered\textsuperscript{148}. These skeletal changes may become severe enough to be classified as crippling skeletal fluorosis.

Probable toxic dose (PTD) in humans has been given a value of $5\text{mg/kg}$ by Whitford\textsuperscript{135}. Exposure to fluoridated products capable of serious acute toxicity is rare. For example, $10\text{ml}$ of mouth rinse ($230 \text{ppm NaF}$) is usually recommended and the amount of fluoride in this volume is well below the PTD, even if it was completely swallowed. As for dentifrices ($1000 \text{ppm}$), $1 \text{g}$ quantities are usually applied. For serious acute toxic dosages to be reached, $50 \text{g}$ of would have to be swallowed by a one year old to reach PTD. Probable toxic doses are not available for animal groups.

There are a wide range of estimates for acute lethal dose of fluoride in humans, from $6-9\text{mg F/kg}$\textsuperscript{149} to $100\text{mg F/kg}$\textsuperscript{150}. However, based on a review of case reports, Hodge and Smith\textsuperscript{151}, suggested a “certainly lethal dose” (CLD) in the range of 5 to 10 g of sodium fluoride in a 70 kg man. This corresponds to 32 to 64 mg F/kg of body weight (equivalent to $\text{LD}_{100}$). Values such as $\text{LD}_{50}$ (median lethal dose), $\text{LD}_{10}$ or the minimum CLD are not known. Only the higher end of lethal dosages, are known in humans
because the only information available are from case reports of accidental poisoning in adults or children and in suicide attempts.

In rats, where a controlled dosage can be given to determine a lethal dose, there have also been a number of different estimates for sodium fluoride. Shourie et al.\textsuperscript{152} reported a 24 hour LD\textsubscript{50} of 36 mg F/kg, whereas Lim et al.\textsuperscript{153} reported 44 mg F/kg. More recent studies by Whitford\textsuperscript{135}, found LD\textsubscript{50} values of 85.5 mg F/kg., while Grunninger et al.\textsuperscript{154}, reported 98 mg F/kg.

2.3.7. Fluoride and Soft Tissues

Fluoride is transported by plasma to all tissues and organs. The rate of delivery is generally determined by the blood flow to the tissues. Therefore, fluoride concentrations are achieved more rapidly in well perfused tissues, such as the heart, lungs and liver. Fluoride is concentrated to high levels in the kidney tubules, so taken as a whole, this organ has a higher concentration than plasma.

2.3.8. Fluoride and Mineralised tissues

Approximately 99% of fluoride in the human body is found in mineralised tissues\textsuperscript{134}. The select affinity of fluoride for mineralized tissues, in the short term, is due to the uptake on the surface of hydroxyapatite crystallites by iso-ionic and hetero-ionic exchange\textsuperscript{155}. In the long term, fluoride will be incorporated into the crystal lattice structure in the form of fluoroapatite or fluorohydroxyapatite.
Concentrations of fluoride in mineralized tissues are variable due to i) the level of fluoride intake, ii) the duration of exposure and iii) inter-related factors such as the stage of tissue development, rate of growth, vascularity, surface area and reactivity of mineral crystallites\textsuperscript{134}. In all mineralized tissues, fluoride levels tend to be greatest at the surface since this is the area closest to tissue fluid supplying fluoride. The distribution and concentration will change with age\textsuperscript{156,157}.

Fluoride is not permanently bound to mineralized tissues. Some superficially located fluoride may be lost by back exchange, back diffusion and migration from the mineral to the surrounding tissue fluid, blood, saliva or plaque\textsuperscript{158-161}. Additionally, some of the firmly bound fluoride within the apatite crystals may be lost as the crystals are destroyed during osteoclastic resorption of bone, cementum and dentine\textsuperscript{162,163}, wear\textsuperscript{164-166} or severe acid erosion.

2.3.8.1. Bone

There is literature suggesting that fluoride can affect bone resorption. In vitro, fluoride decreases the solubility of bone mineral\textsuperscript{167}, and fluoride pre-treatment decreases the bone resorption induced by parathyroid hormone\textsuperscript{168}. On the other hand, Baylink et al\textsuperscript{169,170} reported increased resorptive activity. Boivin and Meunier\textsuperscript{171} suggested that the increased resorption is caused by a fluoride-induced secondary hyperparathyroidism. The long term effects of fluoride on bone resorption seem to differ. In osteoporotic patients treated with sodium fluoride for two to five years, together with calcium and vitamin D, a decrease in bone resorptive activity is seen. Increased resistance to
dissolution by osteoclastic enzymes as well as high fluoride levels liberated by fluoroapatite during the resorptive process, might inhibit osteoclastic activity\textsuperscript{167,172}.

Fluoride is the most potent osteoanabolic agent widely used in the treatment of osteoporosis\textsuperscript{173,174}. Sodium fluoride stimulates bone formation at the cellular\textsuperscript{175,176}, tissue\textsuperscript{177} and organ level\textsuperscript{178}, leading to a positive balance per remodelling cycle, an increase in bone volume and a positive overall calcium balance. Sodium fluoride has been shown to increase the proliferation and differentiation of osteoblasts derived from embryonic chick calvaria\textsuperscript{179}. However, the effects on mature human osteoblast-like cells have been variable\textsuperscript{180,181}.

Lindskog et al\textsuperscript{172} studied the effect of a high dose fluoride bolus on resorbing osteoclasts, in vivo. The experimental model was on 4 day old Sprague Dawley rats, which were injected with an aqueous solution of sodium fluoride (60 mg/kg), and observed at 1, 2, 6, 12 and 48 hours. Histological examination was performed on the osteoclast layer between the developing tooth germ and oral epithelium. They found that a significant drop in resorbing osteoclasts was noted at the 2 hour period, followed by a further drop after 6 hours. After 24 hours, the osteoclastic populations started to increase again and this pattern continued when seen after 48 hours. The actively resorbing osteoclast has a low intracellular pH, which allows the easy penetration of fluoride ions. Consequently, a higher level of intracellular fluoride may be accumulated and lead to toxic effects on various enzyme systems in the osteoclasts. They could not, however, exclude the effect of calcitonin and parathyroid hormones on calcium and phosphorus metabolism, although
the almost immediate cell response suggests a direct action on the osteoclasts by the fluoride administration.

In the orthodontic literature, a recent study by Hellsing and Hammarstrom \(^{182}\) studied the effect of fluoride on orthodontic tooth movement in rats. They found that fluoride reduced the amount of tooth movement by about 50\%, when compared to the control samples. Also, a histological examination showed a statistically significant decrease in osteoclast populations in surrounding alveolar bone. This effect on osteoclasts is in agreement with some of the previous research on the effect of fluoride on osteoclast populations\(^{183,184}\).

2.3.8.2. Cementum

2.3.8.2.1. Human studies

Cementum has been reported to have the highest fluoride content of all calcified tissues, up to five times as much as enamel and three times as great as alveolar bone, with values as high as 4000 – 9000 ppm being reported\(^{185-188}\).

Nakata et al\(^{189}\) studied the effect of age and fluoride exposure on concentrations of fluorine, calcium and magnesium in cementum. Human teeth were extracted from residents in low, optimal and high fluoride areas. The dentists in these areas provided the age, reasons for extractions, and indicated whether the teeth were periodontally involved. Fluoride content in the cementum was found to increase with age. Fluoride was also found in higher concentrations in cervical cementum as compared to apical cementum.
They suggested that the cervical cementum may have an increased fluoride content, due to a greater exposure to topical fluoride exposure in the oral cavity.

Stepnick et al\textsuperscript{190}, studied the effects of age and fluoride exposure on fluoride, citrate and carbonate content in human cementum. They studied extracted teeth from residents living in low (less than 0.22 ppm), optimal (0.8 to 1.2 ppm) and high (3.0 to 5.6 ppm) fluoride areas. The teeth were also divided into 3 age groups; under 20 years, 20 to 40 years and over 40 years. Their samples were the same as the that used by Nakata \textit{et al}\textsuperscript{189}. Their conclusions supported the work by Nakata and co-workers.

Stepnick and Gettleman\textsuperscript{191} studied the micro hardness (Knoop diamond indenter) of human apical cementum when related to fluoride exposure and age. This was a continuing study using the same samples as that published by Nakata \textit{et al}\textsuperscript{189} and Stepnick \textit{et al}\textsuperscript{190}. They found that there was no significant difference in cementum micro-hardness in the samples from different fluoride exposure and age groups.

Studies on fluoride distribution in human teeth have shown that fluoride is most concentrated on the surface and reduces as you go deeper\textsuperscript{192-196}. Also the fluoride in cementum increases as the age of the samples increases\textsuperscript{192,197,198}, suggesting there is a build up of fluoride in cementum due to the continuing exposure to fluoride. Cementum, unlike bone does not undergo continuous remodelling and increases in thickness with age. Nakagaki \textit{et al}\textsuperscript{194}, investigated the distribution of fluoride across dental enamel,
dentine and cementum and suggested that there is a continuous uptake of fluoride by the root dentine and cementum throughout the life of the tooth.

Nakagaki et al\textsuperscript{195} found that, in general, fluoride concentrations were higher in acellular cementum and lower in cellular cementum. They reported that acellular cementum was mostly found on the exterior surface and cellular cementum was found on the interior surface. As fluoride uptake continues to increase with the life of the tooth, the external aspect of the tooth will accumulate more fluoride than the interior. However, in cementum where there is cellular cementum present, once again, the highest fluoride concentrations coincided with the acellular layers. They suggested that there was an inverse correlation between cell density and fluoride concentration, due to the slower formation of acellular cementum in comparison with cellular cementum. Being rapidly deposited, cellular cementum would have less time to accumulate fluoride than the more static acellular tissue\textsuperscript{192}.

There is a general decrease in fluoride content from the cervical to the apical cementum, with the mean cervical fluoride content being greater than that of apical cementum\textsuperscript{190,195}.

2.3.8.2.2. Rat studies

There have been studies on the effect of fluoride in animal samples, but I will be mainly reporting on those being studied in rats, as my experimental model is based on this animal group.
Kato et al\textsuperscript{126} looked at the distribution of fluoride across cementum, dentine and alveolar bone. Twenty-eight, five week old rats were divided into four groups. One group served as the control and was given distilled water. The other three groups were the experimental group and were given 25, 50 and 100 ppm fluoridated water, ad libitum, for 10 weeks. After that period, the rats were euthanised and abrasive micro sampling was performed on the respective tissues. Their results showed that the concentration of fluoride was greatest in cementum when compared to alveolar bone and dentine. The concentration of fluoride was highest at the surface of cementum and decreased towards the interior. With increasing levels of fluoride in the drinking water, the concentration of fluoride increased significantly in both the outer and inner cementum. Comparisons between the left and right molars showed similar results. In each sample, the fluoride concentrations were highest at the pulpal surface of the dentine, decreased to the cementodentinal junction and rose again towards the root surface of the cementum. The surface of alveolar bone contained a higher concentration of fluoride which decreased with depth. The concentration of fluoride was highest in cementum, followed by alveolar bone and dentine. This study showed a similar fluoride distribution profile in rat samples as were found in human tooth samples. The fluoride concentration in the respective tissues also increased with the increased concentrations of fluoridated water.

Kato et al\textsuperscript{199} reported that fluoride concentrations of cementum were increased with an increase fluoride administration (100 ppm fluoridated water, ad libitum) and the fluoride content was highest at or near the cementum surface and decreased towards the interior.
Kondo et al\textsuperscript{200} studied the fluoride distribution in rat molar cementum in relation to age and the fluoride levels in their drinking water. Fifty four rats were used and divided into 3 groups. Six rats were killed at four weeks of age in one group. The second group (n=24) of rats were given distilled water and the third group (n=24) was given fluoridated water (100 ppm). Six rats from groups two and three were killed at ages 6, 12, 24 and 48 weeks. The fluoride distribution in the rats' molar cementum was then analysed by abrasive micro-sampling, to determine fluoride content. The results showed that the fluoride content of cementum increased with age at the surface, but less so in the interior of the cementum. The rats that had fluoridated water had significantly greater fluoride content when compared to the control samples.

Ito et al\textsuperscript{201} studied the effect of different levels of fluoride on cementum distribution and also the effect of stopping fluoride intake. The dosages of fluoride used were 0, 50 and 100 ppm fluoridated water (ad libitum), for the different durations of 4, 13 and 25 weeks. An additional group of rats in the 50 and 100 ppm groups were provided with fluoridated water for either 4 or 13 weeks and then distilled water for an additional 12 or 21 weeks (cessation phase). Their results showed that fluoride concentration was highest on the outer layer and decreased towards the interior. The fluoride concentrations increased with increasing fluoride dosages. The fluoride concentrations increased with increasing duration of fluoride administration. The effect of stopping fluoride administration resulted in a lower fluoride distribution as compared to those samples that did not have a cessation phase. Ito and co-workers concluded that with the cessation of fluoride
administration, there was a reduction of fluoride concentration on the outer surface of cementum.

2.3.9. Fluoride and Root Resorption

There have been a number of studies showing the positive effect of fluoride against root resorption. Likins et al\textsuperscript{1202} postulated that the direct action of fluoride on bone, cementum and dentine changes hydroxyapatite to fluoroapatite, which is more resistant to the resorptive processes. However, the studies have not been in relation to root resorption caused by orthodontic tooth movement. The field of research into the effects of fluoride on root resorption has been in the field of endodontics and dental traumatology.

2.3.9.1. Human studies

Coccia\textsuperscript{7} investigated root resorption of reimplanted permanent incisors. He had a sample of 82 children who had a total of 129 avulsed teeth, aged from 9.5 to 14.5 years of age. The criteria for selection were teeth that had apical root closure, intact alveolus, and reimplantation within 12 hours of being avulsed. The teeth investigated were kept moist in saline solution or treated for five minutes with 2% neutral sodium fluoride. Radiographs of the reimplanted teeth were taken monthly. After root canal therapy was completed, the teeth were radiographed every three months, six months or yearly, depending on patient cooperation. He found greater root resorption in the teeth that were not treated with fluoride. The level of statistical significance increased with time. They concluded that fluoride treatment of avulsed teeth reduced root resorption, and the
benefits were more effective with reimplanted teeth that had a longer extraoral time interval.

Mahajan and Sidhu\textsuperscript{8} also looked at the effect of fluoride on reimplanted teeth. The teeth used were those requiring extractions due to pulpal or periodontal diseases. The patients ranged from nine to fifty-five years of age. Teeth that were reimplanted after immersion in 2\% sodium fluoride had reduced resorption.

2.3.9.2. Animal studies

Shulman et al\textsuperscript{203} and Barbakow et al\textsuperscript{6} looked at the effect of fluoride treatment on reimplanted teeth in monkeys. Shulman immersed Cebus monkeys' extracted incisors and premolars, immersed them in sodium fluoride for 18 hours or fifty-four hours. The teeth were then reimplanted and splinted. Radiographs of the reimplanted teeth were taken to examine resorption rates. Their results showed a positive effect in most of their samples, although the prolonged extra-oral period may have affected their results. Barbakow and co workers\textsuperscript{6} looked at the histologic response of reimplanted teeth that were pretreated with acidulated sodium fluoride (2\% sodium fluoride with 0.1M phosphoric acid). The extra oral period was 30 minutes. The amount of resorption and ankylosis in the teeth pre-treated with acidulated sodium fluoride was the same as that seen in the control series. Therefore, they concluded that prolonged immersion of roots in a 2\% acidulated sodium fluoride solution prior to reimplantation is not recommended.
Bjorvatn and Massler\textsuperscript{4} looked at the effect of fluorides on root resorption in reimplanted rat molars. The fluorides used in this study were 10% stannous fluoride, 1% stannous fluoride and 2% sodium fluoride. The 10% stannous fluoride treatment had a detrimental effect on the healing process. The 2% sodium fluoride solution had a better effect than the 10% stannous fluoride, but the 1% stannous fluoride had the best results in reducing root resorption.

Kameyama et al\textsuperscript{10} studied the effect of fluoride on root resorption caused by mechanical injuries of the periodontal tissues in rats. The mechanical injury involved the insertion of a sterilized flat needle into the mesial gingival margin towards the mesial root surface of the maxillary first molar. This was repeated three times at intervals of 3 hours for a day. There were four groups. The first group received the injury as described. The second group received the mechanical injury and were administered sodium fluoride (25mg/kg) with a gastric tube thrice weekly for three weeks. The third group did not receive any sodium fluoride or mechanical injury. The fourth group was administered sodium fluoride but had no mechanical injury. Their study demonstrated that resorption lacunae were smaller in length and area in the animals that were administered fluoride. They concluded that fluoride suppressed root resorption induced by mechanical injuries of the periodontal soft tissues.

The direct application of fluoride in these studies seems to indicate its protective effects on cementum against inflammatory root resorption. Literature on the effect of fluoride on orthodontically induced root resorption was investigated by Darendeliler et al\textsuperscript{2}. The
aim of their study was to investigate the mineral content and its associated physical properties of cementum against orthodontically induced root resorption. Thirty-six upper and lower premolars were used in this study, with eighteen serving as controls and the remaining as the experimental group. The experimental group of eighteen, consisted of nine who had light buccally directed force (25g) and another nine who had a heavy buccally directed force (225g). After an activation period of 28 to 29 days, the teeth were extracted, and prepared for electron probe microanalysis. This was to determine the mineral content at different sections of the root surface. The minerals studied were calcium, phosphate and fluoride. With regard to fluoride, they confirmed previous studies which looked at the distribution of fluoride in cementum; fluoride content was highest at the cervical region and reduced towards the apices and fluoride content was highest on the surface of cementum and reduced towards the interior. The overall fluoride content in their samples had large inter-individual variability which made interpreting the effect of fluoride on root resorption difficult. They suggested that the fluoride exposure history of their patient samples could not be determined, thereby causing the variability in cemental fluoride content.

2.4. Micro Computed Tomography

On the 28th December, 1895, Wilhelm Conrad Roentgen gave his preliminary report to the Wurzburg Physical-Medical Society of his discovery of a new phenomenon which he later called X-rays. He was studying the phenomena that accompanied the passage of an electric current through a gas of extremely low pressure. He was working in a dark room, when he discovered that the discharge tube while enclosed in a sealed, black carton to
exclude all light, caused a paper plate covered with barium platinocyanide to become fluorescent, even when it was up to two metres away. In subsequent experiments, he found that objects of various thicknesses which were placed in the path of the rays showed variable transparency when recorded on a photographic plate. One of the first radiographs ever taken was that of his wife’s hand, which showed the bones of her hand and the ring that she was wearing. Due to the unknown nature of these rays, he named them X-rays\textsuperscript{204}.

Since then, the science and technology of x-rays have allowed us to view objects (living and inanimate) in a whole different perspective. However, the limitation of conventional radiographs are that they are a two dimensional projection of the object being irradiated. There have been attempts to reproduce three dimensional structures from a two dimensional film, namely laminography or focal plane tomography\textsuperscript{205}. It involves translating an object/subject together with the detecting medium in such a way that only one narrow slice parallel to the translation plane is in focus. The contrast from features outside this slice is blurred and usually disappears from the image. Sharp images are difficult to obtain because of the thickness of each slice and the smearing of images of the plane outside the imaging plane across the image of the plane of interest.

With the advent of digital computers, an approach superior to laminography became possible, called computed tomography. Early in the 1970’s Hounsfield\textsuperscript{206} developed a commercial system for medical imaging. There are a number of constraints imposed by this system of imaging, as the dose of x-rays must be kept to a minimum and the duration
must be limited as involuntary movement by the subject will cause distortions in the image. These considerations need not apply to the imaging of inanimate objects and longer data collection times can be used to improve image quality. New applications of computed tomography were required for resolutions of smaller samples, thereby the creation of micro computed tomography. This area of development could be used in viewing biological structural materials such as calcified tissues. Advances in micro-tomographic imaging in the 1980s were the by product of the demand for improving area detectors for consumer electronics\textsuperscript{207}.

Tomography produces a two dimensional map of x-ray absorption in a two dimensional slice of the subject, thus there is no compression of three dimensional data into a two dimensional plane. This is achieved by taking a series of x-ray projections through the slice at various angles around an axis perpendicular to the slice. From this set of projections, the x-ray absorption map is computed, and by taking a number of slices, a three dimensional map is produced.

There have been four generations of scanners into which the computed tomography literature classifies. The first generation, or pencil beam system, is the simplest arrangement consisting of an x-ray source, a pinhole collimator and a single detector, as found in Hounsfield’s original design. The second generation uses a parallel beam of x-rays, while the third generation uses a flat fan distribution of x-rays. The fourth generation of computed tomography scanning device uses a cone beam geometry, which
is a three dimensional analogue of the two dimensional fan beam geometry\textsuperscript{208}. This type of equipment is well suited for volumetric computed tomography\textsuperscript{207}.

![Diagram of cone beam computer tomography scanner](image_url)

**Figure 1.** Diagrammatic representation of the cone beam computer tomography scanner

The resolution of a two dimensional image is given in terms of pixels. A pixel is a two-dimensional representation of the smallest unit of colour value within an image. This colour value can be a shade of grey or colour. Imaging software used in computed tomography usually assigns the dimensions of a pixel (x and y axis). The number of pixels per surface area gives the resolution of the image. The quality of an image improves with greater resolution, i.e. the more pixels per unit area. In three-dimensional terms, a pixel is no longer valid, and the term voxel is used. A voxel is a three-
dimensional representation of a pixel, except that it has another dimension, ie. depth. This gives a voxel, a volumetric value in space (ie. x, y and z axis).

Current technology limits the use of micro-computed tomography to small, inanimate specimens, such as bone and extracted teeth samples\textsuperscript{209}. Micro computed tomography can be used for investigations without damaging or removing any material. We plan to utilise the SkyScan 1072 desktop x-ray micro-tomograph (SkyScan, Aartselaar, Belgium) to scan our samples. The SkyScan 1072 is a compact desktop system for microscopy and micro-tomography. It consists of an x-ray shadow microscopic system and a computer with tomographic reconstruction software. The system allows making non-destructive three-dimensional reconstruction of the object’s inner structure from two-dimensional x-ray shadow projections. This is a fourth generation scanner with a cone beam x-ray source with a spatial resolution of between 2 and 5 micrometres. The system operates under the Microsoft Windows environment, using Pentium desktop workstations. The recommended sample size is a diameter of 1.5 cm and a height of 3 cm. The sample is placed on a rotating platform, and depending on its proximity to the x-ray beam, there is a magnification factor. A higher magnification gives greater detail. A high resolution Charged Coupled Device with a resolution of 1024 x 1024 pixels detects the incoming x-rays. The information collected by the SkyScan x-ray micro-tomograph is stored and reconstructed to 16 Bit Mapped Picture files with a resolution of 1024 x 1024 pixels. The software package, VGStudioMax v1.2 (Volume Graphics GmbH, Heidelberg, Germany) is used to collate all the axial slices to form a three-dimensional reconstruction of the scanned image. This software package also has the function to remove the bony tissue
around the scanned tooth, which will then allow us to view any defects on the cemental surface. The advantage of this to our experimental model is that it eliminates the necessity for extraction of the rat samples' molar teeth. Due to the size and delicacy of the molar teeth, forceps extraction of the teeth can create defects on the root surface and also fracture the tooth. We can then visualise any defects on the surface of the cementum, indicating a resorptive defect. These crater defects can then be measured and a volumetric calculation of the defect can then be undertaken.
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The Effect of Systemic Fluoride Intake
on Root Resorption
in
Wistar Rats
4.1. Abstract:

Orthodontically induced inflammatory root resorption is a common complication in the field of orthodontics\(^1\). Fluoride has been reported to have a beneficial effect against root resorption in the field of dental traumatology\(^2\text{-}^{10}\). The effect of fluoride on orthodontically-induced inflammatory root resorption has not been investigated. This study has been undertaken to investigate if fluoride has a beneficial effect in reducing the incidence of root resorption.

Thirty two, female Wistar rats, eight weeks of age were separated into four groups. The first two groups are the control groups (n=6, per group). These rats did not undergo orthodontic tooth movement. The other two groups had orthodontic tooth movement (n=10, per group), consisting of an activated 100 grams closing nickel titanium coil (NiTi 10-000-06, GAC International Inc, USA), connecting the mandibular first molar to the incisors. Fluoridated water (100 ppm) was given *ad libitum*, to one control and experimental group. The remaining two groups received deionised water. After two weeks, the animals were euthanased and samples were harvested.

Scanning of the resorption crater was performed using a Micro CT (SkyScan 1072, Aartselaar, Belgium). Software analysis of the scanned samples provided a volumetric measurement of the resorption craters seen on the mandibular molar cementum surface. The results show that resorption sites were found in the control samples, especially in the distal surfaces, which can be attributed to normal physiological tooth drift. The resorption sites were significantly (p<0.05) increased in the groups receiving orthodontic
tooth movement. Fluoride on average reduces the size of resorption craters, but the effect is variable and found not to be statistically significant (p>0.05).

Key words: Fluoride, Root resorption, 3D Micro CT, Volumetric analysis, Physiological tooth drift, Orthodontic tooth movement, Wistar rats
4.2. Introduction

Fluoride is one of the most common agents used in modern dentistry. In the 1930s a startling link was found in communities with a naturally fluoridated water supply and on average a reduction of up to 50% in caries experience$^{11,12}$. Since then, fluoride has been incorporated into a number of topical dentifrices, such as mouthwashes$^{13}$, toothpastes$^{14}$, topical gels$^{13}$, and also in our water supplies$^{12}$. Fluoride, being the most electronegative element in the periodic table reacts readily to form ionic crystals$^{15}$. The inorganic component of the calcified structures in the human body is composed of hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$). Fluoride readily reacts with and replaces the hydroxyl (OH) group of the hydroxyapatite crystal to form fluoro-apatite$^{16,17}$. Fluoro-apatite has a bigger crystal structure and is more resistant to demineralisation$^{18,19}$. This is the reason why topical fluoride application works in preventing caries formation and the demineralisation of enamel. Fluoride has been shown to be incorporated into the mineralized structures of not only enamel, but also in bone, dentine and cementum$^{20}$. In fact, animal$^{21}$ and human$^{22}$ studies have shown that fluoride is found at its highest concentration in cementum, when compared to the other surrounding calcified tissues. In addition, fluoride in cementum increases with age$^{23-30}$ and is directly related to the concentration of fluoride in the water consumed$^{27,29}$.

The mineralized tissues of the permanent dentition are not normally resorbed$^{31}$. They are normally protected in the root canal by dentine and odontoblasts, and on the root surface by cementum and cementoblasts$^{32,33}$. If the cementum is mechanically damaged, multi-nucleated cells will arrive near the surface and resorption will occur$^{34}$. In the field of
orthodontics, this process has been termed, Orthodontically Induced Inflammatory Root Resorption (OIIRR)\textsuperscript{35}. This process is an unavoidable consequence of orthodontic tooth movement. Studies done in animals by Brudvik and Rygh\textsuperscript{36-39} have shown that OIIRR is part of the hyaline zone removal process. During the removal of the hyaline zone, the cementoblast layer which covers the cementum’s surface can be damaged\textsuperscript{38,39}. This exposes the dense mineralized layer of cementum.

There is a vast amount of published literature showing the beneficial effects fluoride has on reducing the solubility of dental enamel\textsuperscript{12-15}. Our hypothesis is that fluoride incorporated into cementum will change the physical properties of cementum in such that it is more resistant to the resorptive processes during orthodontic tooth movement. The human model does not permit one to medicate our subjects with a higher dose of fluoride than that normally taken in from our fluoridated water supplies and food intake. A rat model was chosen to control the administration of fluoride and previous studies have also shown the similarities of fluoride incorporation into cementum in both humans and rats. The study was undertaken to investigate the effects of orthodontic tooth movement on orthodontically induced inflammatory root resorption, and if fluoride had any beneficial short-term beneficial effect on root resorption.
4.3. Materials and Methods

Thirty two, 7 week old female Wistar rats were used in this study (Ethics Approval, Westmead Hospital Animal Ethics Committee – 134.12-03). The rats were allowed one week to acclimatise to their new laboratory environment. The rats were randomly divided into four groups, two experimental groups (n=10, each) and two control groups (n=6, each) (*Figure 1*).

**Group 1**

Group 1 (n=10) had orthodontic tooth movement but was not fluoridated and received deionised water, *ad libitum*.

**Group 2**

Group 2 (n=10) had orthodontic tooth movement and was fluoridated. They received fluoridated deionised water (100 ppm), *ad libitum*.

**Group 3**

Group 3 is a control group (n=6) and, therefore, did not have orthodontic tooth movement. This group was not fluoridated and only received deionised water, *ad libitum*.

**Group 4**

Group 4 (n=6) is another control group and did not have orthodontic tooth movement, but received fluoridated water (100 ppm), *ad libitum*. 
4.3.1. Fluoride delivery

The rats were fluoridated through their water intake. Sodium fluoride was dissolved into MilliQ (deionised water) to a concentration of 100 ppm. Water was given ad libitum. Every 2 to 3 days, the water was monitored and replenished when necessary. The rats were divided into groups of three or four per cage. At the end of the experimental period, total water consumed by the rats was calculated and then averaged. A daily and total fluoride intake could then be determined.

The rats not receiving fluoride were given deionised water, ad libitum.

4.3.2. Orthodontic tooth movement

Orthodontic tooth movement was implemented by use of a Nickel Titanium closed coil (NiTi 10-000-06, GAC International Inc, USA), with a force of 100g. This protocol is a modification of that used by Ashizawa and Sahara. The NiTi coil was ligated to the rat’s left first mandibular molar by means of a silk suture. The coil was then activated to produce a continuous force of 100g and then ligated to the mandibular incisors with steel ligatures (Figures 2a & b).

Attachment of the nickel titanium coils to the rat’s mandibular teeth was performed under anaesthetic. Initially the rats were administered a gaseous anaesthetic agent (halothane). This sedates the rats to an appropriate state whereby they could be weighed. An appropriate amount of anaesthetic agent was injected into the peritoneum. The anaesthetic agents used were Xylazine (10mg/kg) and Ketamine (90mg/kg).
The experimental period was 2 weeks. After this period, the rats were euthanised using carbon dioxide. The mandibles were then dissected and sectioned mesial and distal to the left first mandibular molar. The samples were stored in MilliQ (deionised water)\textsuperscript{42}.

### 4.3.3. SkyScan 1072 Micro CT (SkyScan, Aartselaar, Belgium)

The samples were scanned with a SkyScan 1072 Micro CT (SkyScan, Aartselaar, Belgium, [www.skyscan.be](http://www.skyscan.be)). The SkyScan 1072 Micro CT is a compact, desktop x-ray system for the non-destructive three-dimensional reconstructions of samples, with a high spatial resolution. It obtains transmission images and reconstructs cross sections into three-dimensional images for further analysis. The software used for this reconstruction is provided by SkyScan, ConeRec v2.13 ([www.skyscan.be/next/downloads.htm](http://www.skyscan.be/next/downloads.htm)). The SkyScan 1072 is very similar to the Computed Axial Tomography equipment used by radiologists for medical purposes. The recommended sample size is a diameter of 1.5cm and a height of 3cm. The sample being investigated is placed on a rotating platform in front of the x-ray source (*Figure 3*). Depending on the proximity of the samples to the x-ray source, the computer indicates the magnification factor. The closer the x-ray source is to the sample, the greater the magnification and the higher the detail. The SkyScan 1072 is able to provide a resolution of 2 microns per pixel at maximum magnification. A high resolution CCD (Charged Coupled Device) with a resolution of 1024x1024 pixels will detect the x-rays from the samples scanned and the data collected are stored. Throughout the scanning procedure, the samples are rotated 180 degrees and depending on the radio-opacity of the samples, a scanning time is selected per degree of rotation. A
scanning period of 2 seconds per degree of rotation was found to provide the best contrast and image quality. The average scanning time per sample was around 2 hours.

4.3.4. Cone Beam Reconstruction

The raw data collected needed further reconstruction to provide an axial picture (Figure 4) cross section. This was achieved through the software provided by SkyScan, ConeRec v2.13. Around a thousand cross-sections were collected per sample and the time taken for the reconstruction was approximately 6 hours. Once this has been completed, the raw data were converted to 16 bit, Bit Mapped Picture files, with a resolution of 1024x1024 pixels.

4.3.5. VGStudioMax v1.2 (Volume Graphics GmbH, Heidelberg, Germany) v1.2

This is the software package used to reconstruct the data collected into a viewable three-dimensional reconstruction of the samples into images of 256 shades of gray. The scanned images include both the mandibular molar and the surrounding bone structure (Figure 4). Using software, the bone is removed from the images, leaving the tooth for further analysis, ie. software extraction. The rat’s mandibular molar consists of 4 roots (mesial, distal, lingual and buccal). We analysed the mesial and distal roots. The segmented roots (mesial or distal) is analysed separately. From here the three-dimensional image of the tooth (Figure 5a) can be manipulated (eg rotated) to allow for visualisation of resorption craters (Figure 5b & c). Once the resorption craters are located, they can be further isolated using software.
4.3.6. Chull2d

All images stored are as cross-sections of the total sample. A software package like VGStudioMax gathers all the cross-sectional images and reconstructs them as a three-dimensional picture. For a volumetric analysis of the crater images, a convex hull software was written by Dr. Allan Jones. The software assumes that the surface of the tooth is convex in nature. When a crater is present, there is a break in the convexity. The software (Chull2d) will reconstruct the loss of convexity and connect the 2 points at the edge of the break, and calculates the area \((Figure\ 6a\ &\ b)\). When it has completed the analysis of all cross-sections, it can then provide a volumetric calculation of the crater defect.

4.3.7. Statistical Analysis

Statistical analysis was calculated using SPSS v12 (SPSS Inc, Chicago, Illinois). Univariate analysis of variance and pairwise comparisons between the different groups were performed. Bonferroni adjustments were done for multiple comparisons due to the small sample sizes.

4.4. Results

Virtual extraction of the tooth samples via software was performed for two main reasons. The small size of the molar made actual physical extraction exceedingly difficult and the chances of tooth or root fracture were high. Secondly, physical extraction may create additional defects on the root surface and this may mar our results.
4.4.1. Weight

The average weight of rats at the start of the experiment for groups 1, 2, 3 and 4 were 225 ± 13g, 228 ± 19g, 219 ± 23g and 221 ± 13g respectively. All rats that were anaesthetised and had orthodontic appliances placed (groups 1 and 2) had an initial weight loss, but the weight increased with time. However, at the end of the 2 weeks, on average, group 1 lost 16g and group 2 lost 28g. The control groups, that did not undergo orthodontic tooth movement, both increased in weight, with 32g in group 3 and 21g in group 4.

4.4.2. Water consumed

The water consumed by the rats were measured every 2 to 3 days and replenished when necessary. On average the rats receiving deionised water consumed about 16ml per day. The rats receiving fluoridated water (100 ppm) consumed on average 10ml per day.

4.4.3. Fluoride intake

Sodium fluoride consumed by the rats on average, calculated from their daily water (100 ppm) intake is 1mg per day.

4.4.4. Effect of orthodontic tooth movement on crater size

The mean crater volume of the teeth that had orthodontic tooth movement was greater than the control samples (Table 1). A Univariate Analysis of Variance comparing the total volume of craters with the different groups, showed that orthodontic tooth
movement demonstrated a statistically significant increase in the size of the craters (p<0.05) (Figure 7, Table 2). This was seen when comparing Group 1 with the control groups (groups 3 and 4). Further comparisons used the cube root volume rather than volume. Assumptions for ANOVA models were better satisfied on the cube root scale, particularly the assumption of equal variability. ANOVA analysis (cube root volume) showed that once again, orthodontic tooth movement showed significant effect in the size of craters (Figure 8, Table 3). The samples which had orthodontic tooth movement (with and without fluoridated water) was found to be statistically significant when compared to both control groups.

4.4.5. Effects of fluoride on crater size

We compared the groups that received fluoride to the ones that did not. When comparing Groups 1 and 2, that is when both groups had orthodontic tooth movement but only one had the fluoridated water, there was no significant difference found (p>0.05). When comparing Groups 3 and 4, the control groups receiving no orthodontic tooth movement, once again, no statistical significance was found (p>0.05) (Figure 7, Table 2). Using ANOVA of cube root volume, there was no statistical significance found between the control groups. There was an improvement when comparing the group receiving orthodontic tooth movement and cube root volume, but this was only slight and the result was still statistically insignificant (Figure 8, Table 3).
4.5. Discussion

There are numerous studies investigating Orthodontically Induced Inflammatory Root Resorption (OIIIRR). The causes of OIIIRR have been factors such as genetic predisposition\(^{43-46}\), age\(^{47-53}\), gender\(^{43,47}\), tooth vitality\(^{54-57}\), tooth anatomy\(^{43,53,56,58-61}\), nutrition\(^{62}\), habits\(^{43,59,63}\), previous trauma\(^{64}\), magnitude of orthodontic force\(^{65-67}\), treatment mechanics\(^{56,68,69}\), direction of tooth movement\(^{70,71}\) and duration of treatment\(^{43,56,72}\). Knowing these factors, efforts have been made to prevent or reduce the incidence of root resorption with approaches such as reducing the duration of treatment\(^ {73} \), use of light forces\(^{49,66,74}\), habit control\(^{43,63}\) and thorough medical and familial history\(^{43-46}\). Pharmacological agents have been tested as well. The effect of L-thyroxine was found to be beneficial in reducing root resorption by 50% in the samples tested by Loburg and Engstrom\(^{75}\). However, the sample size was small (n=3) and the prescribed dose was arbitrarily chosen. Shirazi et al\(^{76}\) demonstrated that increased dosages of L-thyroxine reduced the extent of root resorption and increased the amount of tooth movement, indicating that this hormone might also have an effect on bone resorption. Christiansen\(^{77}\) also suggested this effect with regard to Loburg and Engstrom’s results. Bisphosphonates were also found to have an effect on root resorption. Bisphosphonates are commonly used in the treatment of osteoporosis, because they are potent inhibitors of bone resorption\(^ {78,79} \). Various studies have indicated the beneficial effects of bisphosphonates in reducing root resorption\(^ {90-82} \) but other studies have shown that it may increase the resorptive pattern instead\(^ {83,84} \). Corticosteroids also seem to affect root resorption, but are dose dependant\(^ {85,86} \).
Fluoride, being a very commonly found trace element in our water supply and foods, is also readily absorbed by our body’s gastrointestinal tract\(^\text{87}\). Once in the body, fluoride is transported to the hard and soft tissues via the plasma. Fifty percent of the fluoride will be excreted within 24 hours through the renal system\(^\text{88}\). Almost all of the remaining fluoride will be retained by the calcified tissues within the body\(^\text{89}\). In our rat sample the approximate amount of fluoride retained from their consumption of fluoridated deionized water is approximately 0.5mg. The acute lethal dose of sodium fluoride found in rodents, range from 36mg/kg to 85.5mg/kg\(^\text{89}\). The dosage given in this study is approximately 0.11 mg/kg per day. Once absorbed, it readily interacts with the body’s mineralised tissue to form a strong and stable ionic structure called fluoro-apatite. This form of hydroxyapatite is more resistant in terms of its solubility\(^\text{16-19}\). Studies in man and in rodents have shown that the fluoride concentrations found in cementum rises proportionally to the amount of fluoride consumed\(^\text{27,29}\). Fluoride\(^\text{21,22}\) is found in the highest concentrations in cementum when compared with alveolar bone, cancellous bone, dentine and enamel respectively. The amount of fluoride found in cementum increases with age\(^\text{23-29}\) and is more concentrated at the surface\(^\text{21,23,24,90}\).

There has been a vast amount of literature published on the effects of fluoride on the mineralized structures of teeth. However, with regard to the effects of fluoride on root resorption, there have been relatively few studies. Most of the studies have dealt with traumatically avulsed teeth; particularly susceptibility to inflammatory root resorption, and resorption being inhibited by topical application of a fluoride solution prior to reimplantation. A recent study by Darendeliler et al\(^\text{67}\) investigated the mineral content of
cementum (calcium, phosphate and fluoride), and the effect upon the physical properties of cementum. They postulated that the mineral content of cementum would affect its physical properties, thereby changing its resistance to orthodontically induced root resorption. The fluoride content of cementum had high inter-individual variability, most likely due to the different fluoride exposure of their patient samples. Due to this variability, they were unable to correlate the fluoride content of cementum with its resistance to resorption.

4.5.1. Methodology

This study aimed to investigate the influence of fluoride on root resorption. An animal model was chosen to have better control of the variables and reduce other influencing factors. Human subjects were rejected due to their variability of fluoride intake. Wistar rats are bred to be genetically the same, thereby eliminating genetic variables. All samples used were of the same age at the start of the experiment (8 weeks), and the development of their teeth and surrounding structures should have been completed as suggested by Matias and co-workers.91

The samples were divided into four groups, two of which were controls. It has been previously reported that teeth normally undergo physiological root resorption and that this is possibly due to distal migration.92 The aim of the present study was to investigate the amount of normal physiological root resorption, the effect of orthodontic tooth movement on root resorption, and if fluoride had any effect on the amount of root resorption. The experimental groups had orthodontic tooth movement of their
mandibular first molar, to initiate root resorption. The orthodontic tooth movement model was adapted from Ashizawa and Sahara\textsuperscript{40}, but has also been used by other researchers in the field such as Brudvik and Rygh\textsuperscript{36-39,93,94} and Mavragani et al\textsuperscript{95}. The only difference being that we used the mandibular molars rather than the maxilla, to conform to previous resorption studies by our department\textsuperscript{96}. A nickel titanium coil (NiTi 10-000-06, GAC International Inc, USA) was used to produce a continuous force\textsuperscript{41} over the experimentation period of two weeks. The orthodontic tooth movement will start the process of orthodontically induced root resorption and because of the continuous nature of the force applied, cemental repair should not occur. Owman Moll and co workers\textsuperscript{97} showed cementum repair as soon as 2 weeks after stopping orthodontic tooth movement. This methodology allowed us to study the maximum resorptive sites after the 2 week experimentation period, without the possibility of the reparative phase.

Fluoride administration is best achieved by absorption via the gastrointestinal tract. Deionised water was mixed with sodium fluoride to a concentration of 100ppm\textsuperscript{21,28,29}. Hellings and Hammarstrom\textsuperscript{98} looked at resorption sites using a scanning electron microscope and found definitive resorption sites forming within a week of appliance placement. Our 2 week experimental period would be sufficient to produce resorption sites for analysis.

Analysis of the resorption sites in our samples with the use of the SkyScan 1072, was performed due, to its non-destructive x-ray computer tomography scanning properties. Samples scanned were of the rats’ mandibular first molar and surrounding bony tissue.
Using a three-dimensional graphical software (VGStudioMax v1.2) we removed the surrounding bone. This process was advantageous because no further preparation of our samples was necessary and the physical extraction process could create artifacts on the surface of the cementum being analyzed. The scanned images were of a three-dimensional quality and, therefore, a volumetric analysis of the resorption craters could be assessed. Previous volumetric analysis by Chan et al\textsuperscript{99}, using a scanning electron microscope with software analysis had been done but the images have always been two-dimensional in quality. Software (Analysis) interpretation of the grey scale values within the confines of the crater determined its depth and therefore a volumetric measurement was inferred.

4.5.2. Resorption Craters

4.5.2.1. Control groups

When looking at both the control samples, even when there is no orthodontic tooth movement, resorption craters are still found. An explanation for this may be the effect of tooth drift and the resorptive changes in bone, periodontal ligament and cementum during this process. A study by Dastmalchi et al\textsuperscript{100}, using human teeth, suggested that the tensional forces of normal physiological tooth movement causes an increase in cementum deposition. They found statistical significance when comparing the thickness of cementum in the distal surface to that of the mesial surface. Dastmalchi et al\textsuperscript{100} postulated that the tensional forces on the distal cementum surface increased the formation of cementum, thereby increasing its thickness. Rat molars drift distally under physiological conditions\textsuperscript{101-103} because the bony alveolar walls surrounding the roots
undergo continuous formation on the mesial side and active resorption on the distal side. A recent publication by Kimura et al., investigated the effect of physiological drift on rat dental root resorption. Histological examination of the tissues using TRAP staining demonstrated cellular activity in the distal surface of the rat molar tooth. Electron microscopy of the distal cementum surface showed active resorption, peaking around the age of 5 to 6 weeks, and with time, the reparative phase as well. In our control samples, resorptive sites were found on the distal surface. Only one sample had a resorption crater on the mesial surface. These distal resorption sites can be explained by the normal physiological drift of teeth (Table 7).

4.5.2.2. Experimental groups (Orthodontic tooth movement)

There is a dramatic increase in crater volume when comparing the controls with the experimental group. The effect of orthodontic tooth movement is shown to have a dramatic effect on the crater volume. Previous researchers have shown the dramatic effect of orthodontic tooth movement on the incidence of root resorption. Our results showed that orthodontic tooth movement is statistically significant (p<0.001) with the incidence of root resorption, and this is in agreement with the available published literature.

4.5.2.3. Experimental groups (Fluoride)

The effect of fluoride on root resorption crater volume seems to be initially encouraging. The average crater volume is smaller by almost half, when compared with the groups that did not receive fluoride in their drinking water. In the control samples, the mean volume
of resorption craters found in the fluoridated group was about 62% of the non-fluoridated group. In the experimental groups that had orthodontic tooth movement, the fluoridated group had craters about 54% of the volume of the non-fluoridated group. The mean crater volume in both fluoridated groups was less when compared to their corresponding non-fluoridated samples (Table 1). However, when we analysed the data statistically, a different picture emerged. Due to the large range of results from our samples, the effect of fluoride on root resorption was found not to be statistically significant (p>0.05) in the experimental groups and of no statistical significance at all in the control groups (p=1.000) (Table 3).

The effect of fluoride on orthodontic tooth movement was investigated by Hellsing and Hammarstrom\textsuperscript{104}, who found that fluoride reduced the number of osteoclasts, thereby reducing the amount of tooth movement in their rat samples.

It is possible that the reduction in osteoclasts reduces bone remodelling leading to an increase in the hyalinization zone, which may amplify the resorptive processes. This may be the reason why cementum with higher fluoride content may still undergo just as much resorption.

The effect of fluoride on bone metabolism has been well reported in the medical field, as it has been used as a drug by itself or as an adjunct, in the treatment of osteoporosis\textsuperscript{105}. Fluoride treatment, results in an increase in bone mass as a result of the stimulation of bone formation\textsuperscript{106}. There is some evidence that fluoride decreases the solubility of bone
and bone resorption\textsuperscript{107}. Sodium fluoride stimulates proliferation and differentiation of osteoblasts\textsuperscript{108}. Its exact effect on bone formation is complicated and largely unknown, and it has a narrow therapeutic dosage\textsuperscript{103}. Coupling fluoride with Vitamin D\textsuperscript{109}, calcium\textsuperscript{110}, bisphosphonates\textsuperscript{111} and other supplements has been reported in the medical literature in the treatment of osteoporosis with varying success\textsuperscript{112}. Published literature has also shown that factors such as predisposing systemic conditions (especially bone metabolic disorders)\textsuperscript{62,113-117} and associated medications\textsuperscript{75,76,80-86} have an appreciable effect on the amount of root resorption found.

Recently, Kale et al\textsuperscript{118} looked at the effect of 1,25 dihydroxycholecalciferol on orthodontic tooth movement and found that it increased tooth movement. 1,25 dihydroxycholecalciferol is a biologically active Vitamin D derivative which induces the differentiation of osteoclasts, and increases the activity of existing osteoclasts. Kale suggests that this process increases the resorption of bone and promotes tooth movement. However, recent literature seems to indicate that Vitamin D and its metabolites are effective in the treatment of osteoporosis\textsuperscript{109}.

An increased fluoride intake has been shown to induce secondary hyperparathyroidism\textsuperscript{119}. Fluoridated bone is more resistant to resorption and therefore the availability of calcium within bone is reduced. Parathyroid hormone’s main action is to mobilize calcium from bone. Hyperparathyroidism has been shown to increase the risk of root resorption\textsuperscript{120}. 

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The metabolism of physiological bone and cementum remodelling is complicated and the present literature is unable to completely explain the interaction between the two hard tissues. Fluoride has an effect on both bone and tooth structures. This dual effect is possibly the reason why our results show such variability. Even though fluoride has been shown to be more concentrated in cementum than the surrounding alveolar bone\textsuperscript{21,22}, its exact effect on the remodelling and resorptive process of these tissues needs further clarification.

4.6. Conclusion

The results of this study show that

1. Teeth not undergoing orthodontic tooth movement have resorption craters due to normal physiological tooth drift. These craters are found mainly on the distal surfaces.

2. Orthodontic tooth movement has a significant effect on the volumetric measurements of root resorption craters. These craters are much larger and extensive when compared to control samples.

3. Fluoride seems to have a variable effect on the volumetric quantification of root resorption craters. While on average, the resorptions sites are smaller when fluoride is administered, the variability and range of sizes found makes this effect statistically insignificant. The beneficial effect of fluoride on reducing cementum solubility may be counteracted by its anabolic effect on bone mass.
4.7. REFERENCES


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4.8. Figures and Tables

Figure 1 Experimental Design
Figure 2a, b Orthodontic Appliance in Rat’s mandible
Figure 3 Cross sectional scan of Rat’s mandible
Figure 4a Three dimensional reconstruction of rat’s molar root
Figure 4b, c Three dimensional reconstruction of resorption craters
Figure 5a, b Convex hull analysis program
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Figure 6 Box plot graph of resorption craters (volume) in the different experimental groups
Table 2 Analysis of Variance (Total Volume) of the different experimental groups
Figure 7 Box plot graph of resorption craters (cube root volume) in the different experimental groups
Table 3 Analysis of Variance (Cube Root Volume) of the different experimental groups
Table 4 Crater volumes from rat samples (voxels)
Table 5 Crater volumes from rat samples (mm$^3$)
Table 6 Crater volumes found on Mesial surface of rat molar roots (voxels)
Table 7 Crater volumes found on Distal surface of rat molar roots (voxels)
Table 8 Crater volumes found on Lingual and Buccal surfaces of rat molar roots (voxels)
Figure 1. Experimental Design

- **Group 1**: Fluoride, n=10
  - 2 weeks activation 100g from NiTi coil
  - Euthanized
  - Samples collected

- **Group 2**: No Fluoride, n=10
  - ORTHODONTICS

- **Group 3**: Fluoride, n=6
  - Control
  - ORTHODONTICS

- **Group 4**: No Fluoride, n=6
  - Control

- n=32 (♂♂♀♀) Wistar rats, 8 weeks old
Figure 2a. Diagrammatic representation of the mechanical force applied between the mandibular incisor and first molar.
Figure 2b. Orthodontic appliance insitu: a closed Nickel Titanium coil attached to the mandibular molar and incisor.
Figure 3: Diagrammatic representation of the cone beam computer tomography scanner.
Figure 4. Cross section axial scan of mandibular molar root in surrounding alveolar bone.
Figure 5a. Three dimensional reconstruction of axial scans of distal root of rat's mandibular molar.

Figure 5b & c. Three dimensional reconstruction of craters found on root surface.
Figure 6a. Series of cross sectional scans of resorption crater

Figure 6b. Convex hull analysis
Line across crater defect demarcating loss of surface continuity
<table>
<thead>
<tr>
<th>Sample Groups</th>
<th>Mean Crater Size (x 10^{-4} \text{ mm}^3)</th>
<th>Range (x 10^{-4} \text{ mm}^3)</th>
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<tr>
<td>Ortho</td>
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<td>31 - 128</td>
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<tr>
<td>Ortho &amp; Fluoride</td>
<td>42.5</td>
<td>4.9 - 143.1</td>
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<tr>
<td>Control</td>
<td>8.1</td>
<td>0.02 - 18</td>
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<tr>
<td>Control &amp; Fluoride</td>
<td>5</td>
<td>1.2 - 14</td>
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Table 1. Mean crater volumes – average of total crater volumes on mesial and distal roots
Orthodontic Tooth Movement

Figure 7. Box plot comparing the crater volume in rats that were fluoridated (green) and non fluoridated (red), with those receiving orthodontic tooth movement (Ortho.) and those that did not receive orthodontic tooth movement (Controls).
## Pairwise Comparisons

Dependant Variable: TOTVOL  Total crater volume mm³x1000

<table>
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<tr>
<th>(I) GROUP</th>
<th>(J) GROUP</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval for Difference&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
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<td>1 F + Ortho</td>
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<td>.119</td>
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<td>-7.757</td>
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<td>2.573</td>
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</table>

Based on estimated marginal means

* The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

**Table 2: Analysis of Variance of the four groups of rats**
Figure 8. Box plot comparing the crater volume in rats that were fluoridated (green) and non fluoridated (red), with those receiving orthodontic tooth movement (Ortho.) and those that did not receive orthodontic tooth movement (Controls).

Cube Root Volume
## Pairwise Comparisons

**Dependent Variable: Cube Root Total Volume**

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<thead>
<tr>
<th>(I) GROUP</th>
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<th>Mean Difference (I-J)</th>
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<th>Sig. ²</th>
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Based on estimated marginal means

². The mean difference is significant at the .05 level.

³. Adjustment for multiple comparisons: Bonferroni.

### Table 3: Analysis of Variance of the four groups of rats
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Voxels = computer "talk" for a volume unit

Table 4. Resorption crater volumes of rat molar roots (voxel)
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Conversion = converts voxels to real world measurement of cubic mm

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Table 8. Resorption craters of rat molar roots categorised by surface localised – Lingual and Buccal surfaces.
5. FUTURE DIRECTIONS

1. The present study's aim was to investigate if there are any protective qualities induced by a high fluoride intake on orthodontically induced root resorption. The assumption of this study is that a high quantity of fluoride intake, will lead to increased fluoride content in cementum, which should render it more resistant to orthodontically induced root resorption. However, the assumption of this linear relationship may be incorrect (i.e. the greater the intake of fluoride, the greater the resistance to root resorption). Future investigations might study the effects of different dosages (fluoride) on root resorption craters.

2. The administration of fluoride at the start of the experimental tooth movement may not maximise its protective qualities. The timing of the administration of fluoride may also influence the resorption of cementum. A period of 2 weeks, fluoride treatment prior to experimental tooth movement, may change the pattern of root resorption seen in our rat samples.

Additionally, after two weeks of tooth movement, inactivation of the NiTi coil will start the healing process of resorption craters. The administration of fluoridated water during this period may influence the repair of the resorption craters.