A volumetric analysis of root resorption craters after the application of controlled intrusive orthodontic forces in human premolars: A micro CT Scan study.

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A Thesis submitted in partial fulfilment of the requirements for the degree of Master of Dental Science (Orthodontics)

Discipline of Orthodontics

Faculty of Dentistry

University of Sydney

Australia

2004

Dedication

To God who has always helped me.

To my wonderful husband and best friend, Bill. I thank you for your love, patience, kindness, encouragement and unconditional support. My victories are also yours.

To my parents Dea and Paulo. Thank you for your utter love and support. Your words of wisdom always brought me comfort and guidance.

To my family, Duse, Rachel, Adriano, Ana Julia, Therese, John, Catherine and Perry for your incredible help and understanding.

Acknowledgements

Sincere gratitude is expressed to all the following:

Professor M Ali Darendeliler, Head of the Department of Orthodontics, Faculty of Dentistry, University of Sydney for his supervision and assistance during the development of this thesis.

Dr Allan Jones, Electron Microscopic Unit, University of NSW, for his unrestricted help in the use of the Micro Ct scan - Skyscan 1072. Also for developing the Chull2D software that allowed this methodology to be completed.

Dr. Peter Petocz, Department of Mathematical Sciences, University of Technology, Sydney for his assistance with the statistical analysis.

Dr. Paul Bowker, Department of Oral Surgery, United Dental Hospital, Sydney for his assistance in the extraction of all teeth samples.

Professor Lysle Johnston Jr for his assistance with the statistical analysis.

Dr. Om Kharbanda for his guidance in the preparation of the methodology.

The postgraduate students who helped in gathering suitable samples.

To my colleagues, Drs. Matthew Foo, Angelina Zea, Mark Kebsch and Wangira Chutimantskul, for their continuous support and friendship over the last 3 years.

Australian Society of Orthodontists Foundation for Research and Education for their financial support in this thesis.

Declaration

CANDIDATE CERTIFICATE

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

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Table of Contents

LIST OF FIGURES

LIST OF TABLES

Abbreviations

Mx	Maxilla
Md	Mandible
RME	Rapid Maxillary Expansion
RPE	Rapid Palatal Expansion
SEM	Scanning Electronic Microscope
XMT	X - ray microtomography
NiTi	Nickel Titanium
SS	Stainless Steel
Ca	Calcium
P	Phosphorus
F	Fluoride
PDL	Periodontal Ligament
RR	Root Resorption

Abstract

Intrusion is defined as one of the critical types of orthodontic tooth movement in relation to external root resorption. The aim of this prospective randomised clinical trial was to quantify, three - dimensionally, the amount of root resorption present when different intrusive force magnitudes were applied to human premolars and to establish the sites where root resorption is more prevalent after controlled orthodontic intrusion. Fifty-four maxillary first premolar teeth, orthodontically indicated for extraction, from 27 patients (left and right first maxillary premolars from each patient) were intruded for a period of 28 days using buccal and palatal ß-titanium molybdenum alloy (TMA®) 0.017" x 0.025" cantilever springs. The patients were randomly divided into three groups and different levels of force were used for the three different groups: Group 1, heavy force (225 gm) and control force (0 g) on the contralateral side; Group 2, light force (25 gm) and control force (0g) on the contralateral side; and Group 3, light force (25 gm) and heavy force (225 gm) on the contralateral side. After the experimental period, the teeth were extracted under strict protocol to avoid root surface damage and analysed using a micro CT scan X-ray system (Sky Scan-1072. Belgium) and specially designed software for volumetric measurements. The volume of root resorption craters after intrusion was found to be directly proportional to the magnitude of intrusive force applied. The results showed that the control group have fewer and smaller root resorption craters, the light force group have more and larger root resorption craters than the control group, and the heavy force group have the most and the largest root resorption craters of all groups. The mean volume of the resorption craters in the light and heavy force groups was 2 and 4 fold greater than the control group, respectively. These differences were statistically

significant. The mesial and distal surfaces presented the greatest resorption volume with no statistically significant difference between the two surfaces.

1. Introduction

The physiological or pathological loss of root structure is what can be defined as external root resorption. Physiological resorption refers mainly to the resorption of primary teeth roots prior to the eruption of the secondary dentition. Pathological external root resorption may occur following orthodontic tooth movement or traumatic injury to the tooth, during ectopic eruption of adjacent teeth and secondary to pathological phenomenon. It is an unavoidable consequence of orthodontic movement resulting in the loss of root structure and when extending into dentine it is irreversible.

As there is a potential for severe damage during orthodontic force application and a compromised result after orthodontic treatment, there is a need to improve the understanding of the process and the risks, causes and prevention factors involved in root resorption.

Root resorption occurring in connection with orthodontic tooth movement is associated with local over-compression of the periodontal membrane with development of a hyalinized zone. (Reitan, 1951; Kvam, 1973; Nakane and Kameyama, 1987; Tanaka et al., 1990).

Root resorption at the hyalinized zone occurs initially at the circumference of the necrotic hyalinized zone and this process is followed several days later by resorption on the root surface situated beneath the main part of the necrotic zone. Repair in the periphery of the resorption lacunae also takes place during this second stage (Brudvik and Rygh, 1993,1995).

Ghafari (1994) summarized factors that are associated with external root resorption:

- Physiological and orthodontic tooth movement
- Pressure from impacted teeth, tumours or cysts
- Periapical or periodontal infection
- Tooth implantation or reimplantation
- Occlusal trauma
- Metabolic, systemic and idiopathic factors

Root resorption usually does not start immediately after orthodontic force application but remains active once started until the application of force has ceased. (Linge 1993).

Although the orthodontic literature presents several studies that investigated the external root resorption phenomenon, it still remains a challenge for researchers to identify the factors that interfere with the biological system and are responsible for causing resorption after the application of an orthodontic force.

Qualitative evaluation of root resorption craters might not reveal what is needed for the complete understanding of the occurrence of root resorption in orthodontic patients when different controlled force levels are applied to human teeth. Obtaining a three-dimensional quantitative volumetric analysis of root resorption craters, although technically difficult, might lead researchers and clinicians to the next step in evaluating root resorption.

2. Review of the Literature

2.1 Root Cementum

Cementum is a specialized mineralized tissue covering the root surface and has many features in common with bone tissue (Ten Cate 1998; Thilander et al., 2000). It covers the roots of all human teeth.

One of the main functions of cementum is to provide functional tooth support. It anchors the principal collagen fibers of the periodontal ligament to the root surface.

Root cementum has an important adaptive and reparative functions. The responsive features of cementum are responsible for maintaining occlusal relationship and keeping the integrity of the root surface.

During orthodontic tooth movement, the principal active biologic unit is the periodontium (Brezniack and Wasserstein, 1993). Cementum attaches the PDL fibers to the root and contributes to the process of repair after damage to the root surface. Orthodontic tooth movement can be listed as one of the etiological factors contributing to cementum damage.

2.1.1 Characteristics and Development of Cementum

Tooth cementum is a bone-like mineralized tissue secreted by cementoblasts on the surface of root dentin or in some animals on crown enamel (Diekwisch, 2001)

Cementum on the roots of human teeth was first examined in detail by the physiologist, Jan Evangelista Purkinje and his followers Frankel and Rachkow in 1835 and later by the histologist Anders Adolf Retzius in 1837 (Schroeder, 1986).

Cementum is a component of the tooth itself, but belongs functionally to the dental attachment apparatus, that is, the periodontium (Bosshardt and Selvig, 1997).

Cementum has been described as "bonelike" structure (Ten Cate, 1998). However, unlike bone, it contains no blood vessels, has no innervation and continues to grow in thickness throughout life. It does not undergo continuous mineral turnover (Ten Cate, 1988).

More cementum is formed apically than cervically and its thickness shows characteristic variations among tooth groups and tooth surface (Bosshardt, 1994)

At the cemento-enalmel junction (CEJ), cementum is formed as a thin layer approximately 20-50 μm thick that widens to be approximately 150-200 μm at he apex (Ten Cate, 1998).

Cementum formation on the roots of human teeth continues throughout life unless disturbed by periapical or periodontal pathology (Bosshardt and Selvig, 1997). A straight-line relationship between the thickness of cementum and the age of the tooth has been reported, with an average thickness of 0.1 mm at age 20 years to 0.2 mm at age 55 years (Zander and Hurzeler, 1958).

Aproximatelly, 50 – 60 % of cementum by weight is mineralized consisting of hydroxyapatite crystals [Ca_{10} $(PO_4)_6(OH)_2$] with small amounts of amorphous calcium phosphates and the remaining proportions are organic constituents, predominantly collagen (approximately 25%) and water (approximately 15%) (Ten Cate, 1998). The organic component comprises Type I and Type III collagen and various non-collagenous proteins.

Cementum is the product of cementoblasts, cementocytes, and fibroblasts of the periodontal ligament. All three cell-types originate as daughter cells from the ectomesenchymal cell population of the dental follicle proper (Schroeder, 1986). Its formation begins following the onset of root formation and elongation. Root formation is guided by Hertwig's epithelial root sheath (HERS), a collar of epithelial cells derived from the apical prolongation of the enamel organ. (Ten Cate, 1998).

Diekwisch (2001), defines cementum as a dental follicle derived connective tissue that forms subsequent to Hertwig's epithelial root sheath disintegration. Prior to cementogenesis, Hertwig's epithelial root sheath disintegrates and dental follicle cells penetrate the epithelial layer to invade the root surface. Mesenchymal cells from the dental follicle penetrate the Hertwig's epithelial root sheath to deposit cementum. The Hertwig's epithelial root sheath disintegrates prior to any cementum deposition (Diekwisch, 2001).

The only barrier to protect the cementum from resorptive attack of the osteoclastic cells during tooth movement was thought to be the formative cell layer covering it (Tronstad, 1988; Jones et al., 1988). If there is a breach of this protective formative cell layer, the mineralized tissue and the periodontal matrix will come into contact (Jones et al., 1988). Resorption of the calcified dental tissues occurs if osteoclasts obtain access to the mineralised tissue by a breach in the formation cell layer covering the tissue (Tronstad 1988, Jones et al., 1988).

The exact mechanism by which the resorption process is inhibited is unclear, however, several theories exist to explain the resistance of cementum to resorption.

It has been reported that various uncalcified mineralised tissues in response to orthodontic force are more resistant to resorption. This has been demonstrated in precementum (Gold and Hasselgren, 1992), osteoid (Chambers et al., 1984), and predentin (Stenvik and Mjör, 1970)

Andreasen (1988) related cementum's resistance to resorption to the innermost cellular layer of the PDL (cementoblasts, fibroblasts, osteoblasts, endothelial and perivascular cells) and discluded the peripheral part of the PDL (Sharpey's fibres, cementum, precementum and epithelial rests of Mallassez).

Jones and Boyde (1988), however, did not rule out the Sharpey's fibres, precementum, predentin and elements of the organic matrix as participating in resistance to resorption.

2.1.2 Classification of cementum

The classification of dental cementum can be divided according to:

- The time of formation (primary or secondary cementum).
- The presence or absence of cells within its matrix (acellular and cellular).
- The origin of the collagenous fibres of the matrix (intrinsic fibres resulting from cementoblast activity or extrinsic fibres resulting from the incorporation of periodontal ligament fibres (Schroeder, 1986,1993; Ten Cate, 1998).

The following types of cementum have been described:

- Primary acellular intrinsic fibre cementum (PAIFC).
- Primary acellular extrinsic fibre cementum (PAEFC)
- Secondary cellular intrinsic fibre cementum (SCIFC)
- Secondary cellular mixed fibre cementum (SCMFC)
- Acellular afibrillar cementum (AAC)
- Intermediate cementum (IC)
- Cellular Mixed stratified cementum (CMSC)

(Ten Cate, 1998; Schoeder, 1986).

2.1.2.1 Primary and Secondary cementum

There are two distinct cementoblast populations; the first that forms primary cementum and the second population which is responsible for the formation of secondary cementum.

The cementum attached to the root dentin and covering it from the cervical margin to the root apex is acellular and can also be called primary cementum. This type is also covered, in turn, by cellular or secondary cementum; the cells that formed the secondary cementum have become trapped in lacunae within their own matrix very much as the osteocytes come to occupy lacunae in bone. Acellular cementum anchors the periodontal ligament fibre bundles to the tooth; cellular cementum has an adaptive role. (Ten Cate, 1998).

Secondary cementum differs from primary cementum in several points.

- Structural differences in that the cells are incorporated into the matrix
- Phenotypes of cells are different. This is shown by a monoclonal antibody (E11), which stained the cementoblasts and cementocytes of cellular cementum. Cells lining the surface of primary cementum do not stain for this antibody.
- Developmental origin: The origin of primary cementoblasts is from dental follicle whereas the cells associated with the development of cellular or secondary cementum are derived from non-follicular sources.(Ten Cate, 1998).

2.2 Root Resorption

2.2.1 Historic Background and Definition

Becks and Marshall in 1932 defined the term of resorption as the destruction of formed tooth structure. Brudvick and Rygh(1994) defined root resorption as the active removal of mineralised and a thin layer of non-mineralised cementum.

The physiological or pathological loss of root structure can be defined as external root resorption. Physiological resorption refers mainly to the resorption of primary teeth roots prior to the eruption of the secondary dentition. Pathological external root resorption may occur following orthodontic tooth movement or traumatic injury to the tooth, during ectopic eruption of adjacent teeth and secondary to pathological phenomena.

Bates first reported resorption of permanent teeth in 1856. Ketcham (1927; 1929) was the first to report radiographic evidence of root resorption. He tried to determine whether specific features of an orthodontic appliance or technique could be implicated in root resorption.

Inflammation is essential for tooth movement to occur being an important biologic factor behind the root resorption process after orthodontic force application. Therefore, in understanding the biologic process taking place during the occurrence of root resorption, perhaps orthodontic force induced root resorption would be more appropriately termed orthodontically induced inflammatory root resorption (Brezniack, Wasserstein, 2002).

2.2.2 Classification of Root Resorption

Andreasen (1988) classified external root resorption in three different types:

- **Surface resorption** –Self-limiting process involving small areas. Spontaneous repair occurs from adjacent non-affected parts of the periodontal ligament.

- **Inflammatory resorption** – Initial root resorption has reached dentinal tubules of an infected necrotic pulpal or an infected leukocyte zone.

- **Replacement resorption** – Bone replaces the resorbed tooth mass leading to ankylosis.

Tronstad (1988) described two types of inflammatory resorption:

- **Inflammatory resorption** –The predentin or precementum becomes mineralised or, in the case of precementum, is mechanically damaged or scraped off. He further divides it into two categories:

    1. **Transient inflammatory resorption** occurs when the stimulation for resorption is minimal and for a short period. The defect is usually undetected radiographically and is repaired by a cementum-like tissue.

2. **Progressive inflammatory resorption** occurs when the stimulation for resorption is for a longer period and is seen radiographically as a shortening of roots.

- Replacement resorption where the tooth structure is replaced by bone

Ghafari (1994) classified 3 types of external root resorption:

- **Inflammatory resorption** where resorption is a result of an inflammatory response to a specific stimulus. Inflammatory mediators and phagocytic cells colonize mineralised or denuded cemental surfaces and later dentinal tubules or pulpal tissues.

- **Surface resorption,** a transient inflammation in the outlining area of the root surface (cementum and possibly dentin or pulpal tissues), usually followed by repair with a cementum-like tissue. Generally it is undetected radiographically.

- **Progressive resorption:**

    1. **With bone substitution.** This involves replacement of the resorbed tooth with bone and a resultant ankylosis.

    2. **Without bone substitution.** This involves resorption of the tooth without replacement with bone. This appears to be an uncommon outcome.

Brezniack and Wasserstein, (2002) classify orthodontically induced inflammatory root resorption in three categories according to its degree of severity:

- **Cemental or surface resorption with remodelling** – In this process only the outer layers of cementum are resorbed and they are fully regenerated or remodelled.

- **Dentinal resorption with repair (deep resorption)** – In this process, the cementum and the outer layers of dentin are resorbed and usually repaired with cementum material. The final shape of the root after this may or may not be identical to its original form.

- **Circumferential apical root resorption** - In this process, full resorption of the hard tissue components of the root apex occurs, and root shortening is evident. Different degrees of root shortening are possible. When the root loses apical material beneath the cementum, no regeneration is possible.

Root resorption that occurs after application of orthodontic force is generally surface resorption or transient inflammatory resorption. Ankylosis is not a common sequel of orthodontically induced inflammatory root resorption (OIIRR)(Brezniak, Wasserstein, 2002).

According to Brezniak and Wasserstein (1993) surface resorption or transient inflammatory resorption is usually seen after orthodontic treatment with replacement resorption occurring rarely, if ever. However, Tronstad (1988) considers transient resorption as clinically insignificant as does Ghafari (1994). Their classification of external root resorption is as listed above. Orthodontic forces applied to bone and cementum would cause similar resorption; however, cementum is more resistant to resorption thus causing bone resorption leading to root movement.

2.2.3 Incidence of Root Resorption

Henry and Weinmann (1951) in a histological study of 261 permanent teeth from untreated individuals, found that 90% of teeth exhibited histological evidence of root resorption with 76.8% of resorption areas occurring in the apical third of the root, 19.2% in the middle third and 4% in the gingival third.

A certain amount of root resorption may be a normal physiological process, perhaps connected to continuous bone remodeling (Vlaskalic et al., 1998). Numerous studies agree that idiopathic root resorption also occurs in the untreated population; however, its incidence is variable amongst them. Studies also vary in sample size and methodological protocols making it difficult to compare the incidences reported.

Among treated subjects, Mirabella and Artun (1995) found that forty percent of adults had one or more teeth with 2.5 mm of apical resorption or greater after orthodontic treatment. They analyzed standardized periapical radiographs taken before and after treatment of 343 adults representing groups of consecutively treated patients from four orthodontic practices.

Moderate to severe apical root resorptions (>2mm to <1/3 of the root length) have been found in 12-17 per cent of orthodontically treated patients (Linge and Linge, 1991) and excessive root resorption (>1/3 of root length) in 1-5 per cent of the treated population. (Davidovitch, 1996; Levander and Malmgren, 1988)

Kurol et al., (1996) recorded root resorption in 93 per cent of treated adolescents.

Lupi et al., (1997) used periapical radiographs of maxillary and mandibular incisors to measure apical root resorption. They reported from their sample of 88 ethnically and racially diverse adults that 15% of teeth had resorption before treatment and this increased to 73% after at least 12 months of fixed appliance treatment.

2.2.4 Root Resorption Process

The biological mechanism behind root resorption is not fully comprehended. Resorption of the calcified dental tissues occurs when osteoclasts have access to the mineralized tissue through a breach in the formative cell layer covering the tissue (Tronstad, 1988). It can also occur if the pre - cementum is mechanically damaged or scraped off (Tronstad, 1988) or when the mineral and matrix surfaces meet (Jones and Boyd, 1988).

The application of orthodontic force will induce a local process that includes all the characteristics of inflammation (Bossahardt *et al.,* 1998). The inflammation caused after force is applied is essential for the tooth movement to occur, but is also the main component behind the root resorption process. (Brezniack and Wasserstein, 2002).

When force is applied to a tooth, the surface under pressure will undergo periodontal hyalinization. This process usually preceeds resorption as resorbed lacunae associated with hyalinized tissue elimination appear mainly on the pressure side 10-35 days after force application (Rygh 1977). When pressure is below the optimal force of 20 to 26g/cm^2 (Schwartz 1932) resorption ceases.

After initial orthodontic force application, the periodontal ligament goes through an initial morphological change followed by biochemical changes. The histochemical changes seen in the periodontal ligament include: fibre coalescence; hyalinisation (Reitan, 1985); degradation of connective tissue matrices and demineralisation of Sharpey's fibres (Jones and Boyde, 1988). These processes are followed by odontoclastic cells activity.

Brudvik and Rygh (1993) showed that the first cells to be involved in the initial stage of root resorption at the periphery of the necrotic zone were shown to be mono-nucleated, TRAP (tartrate resistant acid phosphatase) negative (non-clast) cells resembling macrophages and/or fibroblasts.

Root resorption occurring in connection with orthodontic tooth movement is associated with local over-compression of the periodontal membrane with development of a hyalinized zone. (Reitan, 1951; Kvam, 1973; Tanaka et al., 1990).

In the later stage of root resorption and beneath the main hyalinized zone, both multi-nucleated, TRAP positive, giant cells without a ruffled border , mono-nucleated, TRAP positive and macrophage-like cells were shown to be responsible for removal of the necrotic tissue and resorption of the surface part of the root cementum (Brudvik and Rygh, 1994).

Brudvik and Rygh (1993, 1994, 1995) confirmed that OIIRR is part of the hyaline zone elimination process. Root resorption at the hyalinized zone occurs initially at the circumference of the necrotic hyalinized zone and this process is followed several days later by resorption on the root surface situated beneath the main part of the necrotic zone. Repair in the periphery of the resorption lacunae also takes place during this stage (Brudvik and Rygh, 1993,1995).

Alteration in levels of cytokines and growth factors may have modified the cellular metabolism, which modulate the activity of both osteoblast and osteoclasts (Centrella *et al,* 1992).

According to Andreasen (1988), resistance to resorption is attributed to the innermost cellular layer of the periodontal ligament which provides protective as well as a repair mechanism to the root surface. Although the precise mechanism is unknown, cells providing such action include cementoblasts, fibroblasts, osteoblasts, endothelial and perivascular cells. Small areas of surface resorption are repaired by the formation of new cementum and periodontal ligament fibres from adjacent vital parts of the periodontal ligament. Larger zones of damage are repaired by ankylosis by cells derived from the alveolar bone and bone marrow.

Jones and Boyde (1988) suggest that the cellular layer covering the root surface such as Sharpey's fibers, cementum, cementoid and Malassez epithelial cells may contribute to resistance to root resorption. Uncalcified mineral tissues, osteoid, precementum and predentine have been reported to be resistant to resorption and to prevent initial loss of root tissue. Continued pressure will lead to root resorption (Reitan, 1985).

Vardimon *et al.,* (1991) found that all resorptions heal as long as the resorbed surface area does not exceed the unresorbed surface area.

The process of repair occurs 35-70 days (Harry and Sims 1982) after force application is removed or is below optimal level. Repair of active root resorption occurs even in the presence of light forces. New mineralised cementum was observed on the resorbed root surface by 21 days (Brudvik and Rygh, 1995).

According to Jones and Boyd (1988), repair takes place by migration of cementoblasts over the resorbed surface.

If clinical treatment is reactivated during active root resorption, the process will continue (Reitan 1974). If appliances are removed and relapse allowed, resorption stops (Reitan 1985, Rygh 1977).

According to Brudvik and Rygh (1994), if active force ends but passive stress of the periodontal ligament remains through retention, resorption continues if necrotic tissue is close to its site. The duration of time of rest needed for repair of lacunae before reactivation of force is still unknown.

2.2.5 Root Resorption Repair

The reparative process has been suggested to begin as soon as the applied orthodontic force is discontinued or reduced bellow a certain level. (Reitan, 1974, 1985; Rygh 1977, 1985; Brudvik and Rygh, 1995).

The deposition of secondary cementum was also described to begin simultaneously with the root resorption process (Stenvick and Mjör, 1970).

Repair is predominantly characterised by deposition of cellular cementum. Most of the reparative cementum is of the cellular type and always covers initially formed acellular cementum. (Barber and Sims, 1981; Bosshardt and Schroeder, 1994; Owman-Moll *et al.,* 1995; Vardimon *et al.,* 1993; Owman-Moll and Kurol, 1998).

Sismanidou and Lindskog (1995) described a healing phase characterised by an initial deposition of acellular cementum within the first two weeks of force cessation, followed by deposition of cellular cementum at more advanced stages of healing. Repair has also been recorded 35-70 days after force application (Stenvik and Mjör, 1970; Harry and Sims, 1982) and during the first week of retention (Owman-Moll *et al.,* 1995).

According to Schwartz (1932), when force decreases below the optimal force (20-26g/cm^2) root resorption ceases.

Owman-Moll *et al.,* (1995) and Owman-Moll and Kurol (1998) examined maxillary first premolars from adolescents after the application of buccal continuous force of 50g, reactivated weekly over a period of 6 weeks. After that, the appliance was made passive for 1-8 weeks (Owman-Moll *et al.,* 1995) and 1-7 weeks (Owman-Moll and Kurol, 1998) The percentage of root resorption and the varying degrees of repair was stated to be 28% after 1 week (Owman-Moll *et al.,* 1995), 35% at 2 weeks (Owman-Moll and Kurol, 1998), 44% at 3 weeks (Owman-Moll and Kurol, 1998), and 82% after 6 and 7 weeks of retention.

Human studies have demonstrated that early repair starts from the centre of the resorption cavity (Barber and Sims, 1981; Owman-Moll and Kurol, 1998).

Owman-Moll *et al.,* (1995) described the following patterns of repair:

1. *No repair*
2. *Partial repair*; part of the surface of the resorption cavity is covered with reparative cementum
3. *Functional repair*; the total surface of the resorption cavity is covered with reparative cementum without re-establishment of the original root contour.
4. *Anatomic repair*; the total surface of the resorption cavity is covered with reparative cementum to such an extent that the original root contour is re-established.

Despite of the large individual variations the results of this study showed that partial repair was the most frequent type of repair during the first 4 weeks of retention (17-31%) and most commonly seen in the cervical third of the roots. Functional repair dominated the healing pattern after 5, 6, 7, and 8 weeks of retention (33-40%) and anatomical repair was most often seen in the apical third. Almost every second resorption lacunae showed resorption surfaces with open dentin tubuli after 8 weeks of retention. There were no large differences in the healing potential in the cervical, middle and apical thirds of the root.

2.2.6 Aetiology of Root Resorption

Root resorption as a consequence of orthodontic treatment has its aetiology related to biological and mechanical factors or a combination of both.

2.2.6.1 Biological factors

2.2.6.1.1 Individual susceptibility and Genetics

Newman (1975) anecdotally has suggested a genetic constituent for shortened roots although the pattern of inheritance was not clear.

Harris (1997) after studying a sample of full siblings (103 pairs), all of whom were treated with the same technique by one orthodontist, demonstrated a substantive genetic factor in susceptibility to external apical root resorption. Heritability estimation was high reaching 70% on average.

It has been established that different degrees of root resorption vary among different people and different teeth. (Vlaskalic *et al*,1998).

Sameshima and Sinclair (2001) after examining records from 868 patients recognized that Hispanic patients are more susceptible to root resorption than Asians or Caucasians.

Al Qawasmi *et al,* (2003) identified linkage and linkage disequilibrium between the interleukin gene *1B* and root resorption in individuals who were treated orthodontically and were part of 35 white American families implicating a substantial genetic component for external apical root resorption.

Furthermore, Al Qawasmi *et al.* (2003) used a sample of 38 American Caucasian families with a total of 79 siblings who completed comprehensive orthodontic treatment to investigate the possible linkage of external apical root resorption with the *TNSALP and TNFRSF11A and TNFa gene loci.* The *TNFRSF11A* gene was found to be associated with external apical root resorption. The association of *IL-1B* and *TNFRSF11A* encoded proteins was demonstrated to act in the same osteoclast formation pathway, which is involved in external apical root resorption associated with orthodontic treatment.

Ngan *et al* (2004), in a retrospective study, analysed pre and post treatment records of 16 monozygotic and 10 dizygotic twins to try to determine the genetic contribution to orthodontic root resorption. Two-dimensional quantitative and qualitative measurements were obtained from panoramic radiographs. Qualitatively and quantitatively determined estimates of concordance for external apical root resorption and overall heritability indicated a genetic component to it. However, the author pointed that a larger sample is needed before models of heritability can be used to determine the components contributing to the variance.

2.2.6.1.2 Asthma and Allergy

In 1996, Davidovitch induced allergic reactions in guinea pigs and applied an orthodontic force against the maxillary molars. The number of osteoclasts was found to be increased in the experimental animals when compared to controls, suggesting that chemical mediators produced in the allergic state may influence cell populations and , consequently, the root resorption process.

Mc Nab et al (1999) in a radiographic study of panoramic films found a statistically significant increase in root resorption in asthmatics after orthodontic treatment when compared to non-asthmatics.

Owman-Moll and Kurol (2000) divided a sample of 50 orthodontically treated individuals in two groups; high risk and low risk for external root resorption based on measurement of the magnitude of root resorption present. They suggested that there might be an association between the extent of root resorption and allergy; however, no statistically significant difference was found between the groups.

2.2.6.1.3 Biological age

Linge and Linge (1983) found that patients who started treatment before the age of eleven had less root resorption than those treated later.

Linge and Linge (1991) also found that there was no evidence for sex or age difference in susceptibility for external apical root resorption after studying possible heritable component for external root resorption in sample of 103 pairs of siblings.

Thilander et al. (2000) suggested that adult patients experienced more root resorption as a consequence of treatment than younger patients.

Sameshima and Sinclair (2001) have examined the records of 868 patients who were treated with full, fixed edgewise appliances. Full-mouth periapical radiographs were used to precisely assess apical root resorption from first molar to first molar in both maxillary and mandibular arches. The results showed that adult patients experienced more resorption than children; however, these results were only substantiated for the mandibular anterior segment.

2.2.6.1.4 Dental age

Rosenberg (1972) reported that incompletely formed roots still reached their normal root length and showed less resorption than those with completely formed roots.

Baumrind *et al* (1996) stated that adults might be more susceptible to root resorption due to a less vascular periodontium ligament.

Hendrix and colleagues (1994) found that posterior teeth showed root shortening during active orthodontic treatment independent of sex, age, and extraction or non-extraction therapy and duration of active treatment. Teeth with incomplete root formation at onset of orthodontic treatment showed root lengthening during active treatment, but did not reach their normal tooth length.

2.2.6.1.5 Individual tooth susceptibility

Brezniak and Wasserstein (1993), after reviewing the literature, reported that the most frequently affected teeth by external root resorption after orthodontic treatment are the maxillary lateral incisors, maxillary central incisors, mandibular incisors, distal root of mandibular molars, mandibular second premolars and maxillary second premolars.

Sameshima and Sinclair (2001) reported that maxillary teeth are more affected than mandibular teeth. They listed the most affected teeth in order of severity being the maxillary lateral incisors, followed by maxillary central incisors, maxillary canines, mandibular canines, mandibular central incisors and mandibular lateral incisors.

2.2.6.1.6 Tooth form and anomalies

Sameshima and Sinclair (2001) stated that maxillary lateral incisors have the highest percentages of abnormal root shapes. They also are the most susceptible teeth for developmental anomalies such as dens invaginatus. This might explain, according to the authors, the high degree of root resorption incidence on those teeth.

They also found that abnormal root shape was a significant factor of root resorption. Dilacerated teeth had the most resorption, followed by bottle-shaped and pointed teeth. These findings are in agreement with previous studies (Mirabella and Artun, 1995; Newman, 1975; Oppenheim, 1942).

Levander *et al.*, (1988) found that the degree of root resorption in teeth with blunt- or pipette-shaped roots was significantly higher than in teeth with normal root form. The pipette-shaped root was shown to be the root form most susceptible to root resorption.

Becker *et al.*, (1991) found that anomalies in the dentition such as ectopic eruption or agenesis were found frequently in patients who had experienced severe root resorption during orthodontic treatment; however, these patients may have required more extensive orthodontic therapy due to the anomaly making definite conclusions difficult.

Lee *et al.*, (1999) investigated the possible significance of dental anomalies as risk factors. Pre-treatment and post-treatment periapical radiographs of 84 patients with presence of at least one dental anomaly and of 84 patients without such anomalies were compared. They found that anomaly did not appear to be associated with increased risk of root resorption.

Kook *et al.*, (2003) assessed periapical radiographs of 114 patients with 60-peg laterals and 54 small laterals before and after orthodontic treatment. Findings showed that there was no statistically significant difference between the peg laterals and normal teeth in relation to susceptibility to root resorption. However a statistically significant difference was found between the small laterals and normal laterals groups.

2.2.6.1.7 Gender

Gender, in general, has not been found to be a factor that influences the incidence or severity of root resorption (Harris *et al* 1997; Sameshima and Sinclair, 2001). Some previous studies report a significant correlation (Newman, 1975 and Baumrind *et al* 1996). However, it is contemplated that these differences might be due to a difference in root maturity between males and females (Dougherty, 1968).

2.2.6.1.8 Presence of root resorption before orthodontic treatment

Harris and Butler (1992) stated that patients showing root resorption before treatment are at risk of loosing more substance during treatment and can be a notable predictor of apical root resorption.

2.2.6 1.9 Habits

Linge and Linge (1991) demonstrated that finger sucking persisting beyond 7 years of age together with tongue dysfunction are significant risks factors for root resorption.

Nail biting (Odenrick and Brattstrom, 1985), tongue pressure and tongue thrust have also been related to increased root resorption (Newman, 1975).

2.2.6.1.10 Previous trauma

Although traumatized teeth show a high incidence of resorption despite the absence of force application, orthodontic treatment can initiate the process of resorption on those teeth. (Andreasen, 1988).

Linge and Linge (1991) found trauma to be a contributory factor for root resorption.

Brin *et al.,* (2000) reported that incisors with a history of trauma that also had been subjected to a course of orthodontic treatment had more extensive resorption than either incisors that had been traumatized without orthodontic treatment, or incisors that had orthodontic treatment without a history of trauma.

Brin and Tulloch (2003) reported no statistically significant difference in the incidence of root resorption on teeth that were traumatized before treatment and those which were not when examining a population of orthodontically treated class II patients.

2.2.6.1.11 Endodontic treatment

Wickwire *et al* (1974) reported a higher incidence and severity of root resorption in endodontically treated teeth.

Authors have suggested that endodontically treated teeth are more resistant to root resorption because of an increased dentin hardness and elasticity (Thilander *et al,* 2000).

Spurrier *et al*, (1990) studied forty-three patients who had one or more endodontically treated incisors before orthodontic treatment and who exhibited signs of apical root resorption after treatment. In each patient the vital contralateral incisor served as a control. The results showed that vital incisors exhibited a greater resorption when compared to endodontically treated incisors. These results are in agreement with a previous study (Reitan, 1985).

2.2.6.1.12 Alveolar bone density and bone turnover

According to Reitan (1974,1985), more resorption occurs with dense alveolar bone than in less dense alveolar bone where a greater number of resorptive cells are associated with decreased marrow spaces. Also a strong continuous force on less dense alveolar bone causes the same root resorption as a mild continuous force on highly dense alveolar bone.

Goldson and Henrikson (1975) found an increase in frequency and severity of root resorption of upper and lower incisors during Stage II of Begg treatment, due to approximation of the apices to the labial compact cortical bone.

Goldie and King (1984) used lactating, calcium deficient rats to produce a decrease in bone density concomitant with an increase in parathyroid hormone and found a reduced amount of root resorption where bone density was decreased.

Vardimon *et al.* (1991), found in monkeys that bone density was only a secondary predisposing factor for root resorption as roots located near the dense buccal cortical plate failed to demonstrate greater resorption than roots near the less dense palatal cortical plate.

Verna and Melsen (2003) studied bone turnover rate associated with external root resorption in 52 6-month-old Wistar rats. Resorption in the lower bone turnover group was higher suggesting that in subjects where a decreased bone turnover rate is expected, the risk of root resorption could be increased.

2.2.6.1.13 Type of malocclusion

Linge and Linge (1991), Harris and Butler (1992) and Hendrix *et al.* (1994), noted that both increased overjet and the amount of root movement correlate positively with the amount of root resorption.

Ghafari (1994) proposed that orthognathic surgery influenced root resorption by affecting the blood supply to the periodontal ligament, bone and/or cementum.

Harris *et al.* (1997) demonstrated that greater ANB values were commonly associated with greater external root resorption.

Brin and Tulloch (2003) found a significant association with the magnitude of overjet reduction and external apical root resorption in a random population of orthodontically treated class II division 1 cases. The authors also suggested that early growth modification treatment that reduces the severity of the overjet might play a role in reducing the likelihood of external root resorption.

2.2.6.2 Mechanical factors

2.2.6.2.1 Type of appliances and different mechanical techniques

Several studies have investigated the correlation between different types of appliances, different mechanical techniques and root resorption.

Kinsela (1971) stated that the light wire Begg technique causes less resorption than edgewise.

Malgreen *et al* (1982) found that there was no difference in root resorption between the Begg and edgewise techniques. They however, found that the frequency of root resorption was significantly higher in traumatized maxillary incisors that were intruded by the Begg technique when compared to the edgewise technique.

Linge and Linge (1983) compared root resorption as a result of the use of fixed and removable appliances. They concluded that fixed appliances are more detrimental to the roots.

Beck and Harris (1994) found no difference in root resorption levels when comparing the Begg and Tweed techniques.

Parker and Harris (1998) found no statistical difference in average external root resorption between the Tweed standard edgewise techniques, Begg light wire technique and Roth prescription straight wire technique.

Blake *et al* (1995) examined pre treatment and post treatment periapical radiographs of 63 patients treated by the Speed and Edgewise techiniques. No statistical difference in the amount of root resorption was reported.

Alexander (1996) compared sectional mechanics with continuous arch wire mechanics and reported no significant difference in the amount of root resorption between the two techniques.

Mavragani *et al* (2000) found the apical root resorption was more prevalent in the standard edgewise group when compared to straight wire group. This was credited to a more efficient force control system of the straight wire system.

Linge and Linge (1991) compared patients treated with full fixed edgewise appliances and activator only patients. They found no resorption in the activator group. They also stated that open activators, plates with clasps and vertical elastics had a very low correlation with root resorption. They also found that treatment with fixed appliances, rectangular arch wires and the use of class II elastics showed increased risk for root resorption.

Linge and Linge (1983) stated that the use of intermaxillary elastics results in jiggling forces and reported significantly more resorption on the side where elastics were used.

2.2.6.2.2 Extraction or Non extraction treatment protocols

McFadden (1989) found no difference in the amount of root resorption present in patients treated with or without extractions. However, Sameshima and Sinclair (2001), found that patients who had four first premolar extractions presented more resorption than those patients who were treated without extractions or with upper premolar extractions only. According to the authors, patients with different extraction patterns (four second premolar extractions, a mandibular incisor or asymmetric extractions) also presented increased resorption.

McNab *et al.* (2000) investigated the association of the appliance type and tooth extraction with the prevalence of apical root resorption in posterior teeth following orthodontic treatment. They reported that non-extraction patients treated with the edgewise appliance demonstrated less posterior external apical root resorption compared with patients in which extractions or a Begg appliance was used.

2.2.6.2.3 Maxillary expansion

Thilander *et al* (2000) found that rapid palatal expansion is associated with root resorption because premolars and molars are compressed against the thin buccal cortical plate during expansion. These findings support evidence proposed by previous studies. (Onderick *et al*, 1991; Vardimon *et al*, 1991)

Sameshima and Sinclair (2001) reported no significant difference in the amount of root resorption with rapid and slow maxillary expansion.

Sameshima and Sinclair (2001) reported no statistically significant difference in the amount of root resorption when comparing slot size (0.018" x 0.022") or arch wire type (stainless steel versus titanium).

2.2.6.2.4 Extent of Tooth Movement

Root resorption occurrence is believed to be directly related to the distance moved by the tooth roots (Dermaut and DeMunck, 1986; Sameshima and Sinclair, 2001).

In a study analyzing the relationship between upper central incisor displacement and external root resorption, Baumrind *et al,* (1996) reported that in orthodontically treated adults, only a relatively small portion of the observed apical resorption could be accounted for by tooth displacement alone.

2.2.6.2.5 Direction of tooth movement

Intrusive forces are reported to be associated with apical root resorption (Reitan, 1974) and together with root torque were found to be the strongest predictors of external root resorption (Parker and Harris, 1998).

De Shields (1969) found no significant associations between vertical movement of the root apex and root resorption.

According to Thilander *et al* (2000), the stress distribution along the roots during bodily movement is less than during tipping.

Rudolph *et al* (2001) investigated the types of orthodontic forces that cause high stress at the root apex using a finite element model. They stated that purely intrusive, extrusive and rotational forces produce stresses concentrated at the apex of the root. The principal stress from tipping movement was concentrated at the alveolar crest. For bodily movement, stress was distributed throughout the PDL. They conclude that intrusive, extrusive and rotational forces produce more stress at the apex. Bodily movement and tipping produce forces at the alveolar crest, not at the apex.

2.2.6.2.5.1 ***Intrusion and Root resorption***

Numerous authors describe intrusion as one of the worst types of orthodontic tooth movements concerning external root resorption. Experimental tooth intrusion performed under controlled conditions has been tested in animal and human studies. Several diverse methodologies have been proposed in the orthodontic literature. Studies presented in the literature can be divided in histological, radiological and scanning electronic microscopic and could be qualitative or quantitative studies. Qualitative studies describe the observations of the author's findings and quantitative studies attempt to measure the amount of root resorption present.

- **Intrusion - Clinical Studies**

In 1970, Stenvick and Mjör analyzed thirty-five premolars that were submitted to intrusive force for a period varying from 4 to 35 days with fixed appliances. The magnitude of intrusive force applied ranged from 35 to 250 grams. An additional untreated 35 teeth served as controls. Histological analysis was carried out after the extraction of the teeth. The authors found mainly pulp changes and circulatory disturbances in the experimental group. The pulp alteration in the experimental teeth could not be directly related to the magnitude of force employed but, according to the authors, there was a tendency towards increased severity with increased force. In the teeth with incomplete root development, disturbances of root formation were frequently encountered. The authors also found external resorption in dentine linked to the magnitude of the force applied and the duration of the force application period. Resorption of the cementum and dentine was found in 60 percent of the experimental teeth. When the force applied was above 100 grams there was less resorption observed than when the force applied was below 100 grams.

Reitan (1974) investigated the initial tissue reaction in the apical portion of roots that have been subjected to movements of varying duration, direction and magnitude of force. The sample consisted of seventy-two human premolars obtained from 32 patients .Non orthodontically treated control teeth coming from 20 patients were used as controls. Intrusive, extrusion and tipping movement was applied with a spring fixed to the first molar and ligated to a twin bracket on the experimental tooth. The forces applied ranged from 25 grams to 240 grams and the experimental periods varied between ten days and forty-seven days. The teeth were all analysed histologically. Root resorption occurred in the majority of cases with all types of tooth movement. For the subjects that were subjected to intrusion, apical root resorption occurred more frequently in the teeth that were intruded with force magnitudes varying from 80 to 90 grams.

Harry and Sims (1982) used Scanning Electronic Microscopy (SEM) to study the effects of different force magnitudes of intrusive orthodontic forces on the topography of the root surfaces. In this qualitative study, they used a sample of 36 maxillary and mandibular bicuspid teeth indicated for orthodontic extractions. Eighteen teeth were subjected to experimental intrusion and the other half were left as untreated controls. Through a partial banded appliance, intrusive force of 50, 100 and 200 grams were applied over intervals of 14, 35 and 70 days. The authors found an increase in the root resorption craters of all experimental teeth when compared to controls. The amount of root resorption increased with longer periods of intrusive force application.

Dermaut and De Munck (1986) developed a radiological study to investigate apical root resorption on the upper anterior teeth. An intrusive arch was issued on the four upper anterior teeth of 20 subjects applying an intrusive force of approximately 100 grams for a period of 29 weeks. An additional untreated 15 subjects served as controls. The amount of intrusion was measured using the lateral cephalogram and periapical radiographs. Their findings describe a root shortening of 18 percent of the original root length. None of the control subjects demonstrated root shortening. This study measured the amount of root length loss in two dimensions.

McFadden (1989) evaluated the relationship between intrusion (25 gm) using utility arches and root shortening. Incisors subjected to intrusive forces showed root shortening on average of 1.84 mm for maxillary incisors and 0.61 mm for mandibular incisors. They did not find any relationship between the amount of root shortening and degree of intrusion achieved. Also, the degree of root shortening was higher in the maxilla than the mandible.

Costopoulos and Nanda (1996) developed a new radiographic method for measuring root length loss. Seventeen patients were treated with a Burstone-type intrusion arch which delivered a level of force of 15 gm per tooth. A control group of seventeen patients in full arch fixed appliances were randomly selected. The experimental period lasted for 4 months. The amount of intrusion and root length loss was measured using lateral cephalograms and periapical radiographs using the crown and root ratios described previously by Linge and Linge (1983). The authors found that the amount of root length loss was not correlated with the amount of intrusion obtained.

Parker and Harris (1998) used orthodontic treatment records of 110 subjects on a mixed longitudinal retrospective analysis to quantify apical and incisal movements of the maxillary central incisor on the sagittal and vertical planes. They used stepwise multivariate linear regression analysis to try to determine which tooth movement is most predictive of external apical root resorption. Forty-one records were from a solo Tweed standard edgewise practice, 29 were obtained from a solo Begg practice and forty were obtained form a solo Roth prescription straight wire practice. Treatment outcome was not considered in the sample selection. The sample consisted of 110 adolescents with similar pre-treatment malocclusions (Class I crowding or bimaxillary protrusion) that were treated with extraction of four first premolars. Lateral cephalograms were analysed at the start, middle and end of treatment to quantify the movements of the most protrusive maxillary central incisor. Fiducial points were located within the bony maxilla to avoid superimposition on external landmarks. There was no statistical difference in average external apical root resorption between sexes or among techniques. Measures of tooth movement were highly predictive explaining up to 90 percent of the variation in root resorption. Apical and incisal vertical movements and increase in incisor proclination were the strongest predictors of external root resorption for each regression model. Incisor intrusion and increase in lingual root torque were together the strongest predictors of external root resorption.

Faltin *et al* (1998) conducted a qualitative scanning electronic microscopy study to evaluate root resorption occurrence after application of continuous intrusive forces of different magnitudes. The sample consisted of twelve upper first premolars that were indicated for extractions. The teeth were divided into three groups: Control, Light force (50 cN) and Heavy force (100 cN) groups. The teeth were intruded for a period of four weeks using specially designed NiTi- Stainless Steel springs. After extractions, the teeth were examined by scanning electron microscopy. It was found that the intruded teeth showed resorptive areas consisting of lacunaes in the mineralised root surface. In the teeth moved with 50 cN showed in the apical third several, in the middle third few and in the cervical third no resorption areas. The teeth that were moved with 100cN, resorptive areas were observed in most of the apical third including the apex contour, several on the middle third and none on the cervical third. In the control group, no resorption was observed. Root resorption was found to be associated with the magnitude of the intrusive force applied.

Faltin *et al* (2001) conducted an ultra structural study of the cementum and periodontal ligament (PDL) using the same sample of teeth that were submitted to controlled orthodontic intrusion in their previous study (1998). They observed signs of degeneration of cell structures, vascular components, and extracellular matrix of cementum and periodontal ligament in all the intruded teeth. More severe changes were observed in the apical region and they were correlated to the magnitude of force applied. The authors suggested a reduction of continuous force magnitude should be considered to preserve the integrity of tissues.

- **Intrusion - Animal studies**

Dellinger (1967) intruded the maxillary premolars of *Macaca speciosa* monkeys using the maxillary first molars as anchorage. The magnitude of force used was 10, 50, 100, 300 grams and the experimental period lasted for 60 days. The direction of tooth movement was measured on pre and post treatment cephalograms. The tissues were then examined histologically to assess damage and tissue response to the intrusive orthodontic movement. The author established that 50 grams of force proved to give optimal intrusion. He also concluded that root resorption was not directly related to the distance the teeth were intruded but was directly related to force magnitude.

Bondevik (1980) studied the pattern of tissue reactions in the periodontium of 55 young 7-9 week old male Wistar rats after controlled intrusion. An additional 5 rats from the same litters were used as controls. The animals were divided into three groups for three different magnitudes of intrusive force application: 0.29 N, 0.49 N and 0.98 N. Animals were sacrificed after 1,3,7,14,21 days and the maxilla, including the teeth, was prepared for histological examination. The results showed that the tissues in the interradicular and apical regions had the typical characteristics of pressures zones; periodontal ligament compression, cell free and semi-cell free zones and initial undermining bone resorption in the earlier phases of force application. Bone and root resorption was noticed in the later phases. The authors also found that the incidence of cell free zones and root resorption lacunae seemed to increase as the force increased.

Lu *et al.*(1999) conducted a qualitative histological and histochemical study to investigate the nature of root resorption resulting from intrusive forces applied to the rat lower molars. Thirty-eight 13 weeks old Wistar rats were used as experimental animals. An additional 12 animals were used as controls. They divided the animals into 3 groups according to the duration of force application that varied from one, two or three weeks. Intrusive force was created using orthodontic bands on the mandibular incisors, a 0.7 mm main wire and a 0.25 mm cantilever spring with helix. The magnitude of the force applied was 50 g .In the control group the springs were not activated. The degree of root resorption and distribution of tartrate resistant acid phosphatase (TRAP) positive cells were evaluated. They concluded that intrusive force of longer duration might lead to a higher frequency in root resorption and that the degree of root resorption activity was higher in the apical area than in the interradicular area indicating that cellular cementum may be resorbed easier during intrusion.

2.2.6.2.6 Continuous and discontinuous force application

There is no consensus in the orthodontic literature whether continuous or discontinuous orthodontic force application leads to an increase in the occurrence of root resorption.

Owman-Moll *et al.* (1995) examined 32 human maxillary first premolars after the application of a buccally directed continuous (24 hours / day) and interrupted, continuous (one week every fourth week) force of 50g over four and seven weeks. They found no difference in the amount or severity of root resorption between the two forces.

Maltha and Dijkman (1996) compared the amount of root resorption after continuous and discontinuous force applications in beagle dogs teeth and found that the extent of root resorption was less when discontinuous forces are used.

Acar *et al.* (1999) examined 22 human first premolar teeth after the application of a 100gm continuous (24 hours/day) force on one side and a discontinuous (12 hours /day) force on the other side during a nine weeks experimental period subjecting the results on patient compliance.

Weiland (2003) analyzed a total of 87 premolars from 27 individuals that were moved bucally by a stainless steel wire (dissipating force) on one side and a super elastic wire (constant force) on the contra lateral side. The initial activation for the super elastic wire was greater than the steel wire but the latter was reactivated every four weeks. The super elastic wire was not reactivated during the 12-week experimental period. The results showed that the amount of movement was greater in the super elastic wire side and so was the amount of root resorption found.

2.2.6.2.7 Treatment Duration

Several studies have reported that the severity of the root resorption is directly associated with the duration of treatment (Reitan, 1974; Sharpe et al, 1987 and Brezniak and Wasserstein, 1993).

Levander and Malgreen (1988) found that 38% of the examined teeth showed apical root resorption after 6-8 months of treatment with fixed appliances.

An apical root loss of 0.9 mm per year during labial root torque has been reported by Goldin (1989).

McFadden *et al.* (1989) stated that treatment time is the most significant factor for occurrence of root shortening after intrusion.

Linge and Linge (1991) showed that duration of treatment using rectangular wires significantly contribute to apical root resorption incidence.

Thilander *et al.* (2000) considered the total duration of treatment to be a more significant factor than magnitude of force in the occurrence of root resorption.

2.2.6.2.8 Force Magnitude

Schwartz (1932) stated that if force application exceeds the optimal level of $26gm/cm^2$, periodontal ischemia would occur leading to root resorption.

Smith and Storey (1952) described the optimal force theory showing that forces between 150-200g produce the maximum rate of tooth movement for distalisation of maxillary canines. Below this range there is little movement, and above this range movement is slowed.

Andreasen and Johnston (1967), Hixon *et al* (1969, 1970) suggested that there is a linear relationship between force magnitude and the amount of tooth movement.

Boester and Johnston (1974) found that the amount of space closure with force magnitude of 140g, 225g, or 310g was about the same but it was significantly less if 55g were used. They found that there was a direct relationship between the magnitude of the applied force and the rate of tooth movement, but once a certain stress level was reached (140g), further increases in stress failed to produce continued increases in the rate of movement. Other studies agree with their finding (Reitan, 1960; Burstone and Groves, 1961; Maltha *et al.*, 1993). Boester and Johnston (1974) also questioned the validation of Smith and Storey's (1952) optimal force theory criticizing the absence of statistical analysis and the canine retraction mechanism. They examined Smith and Storey's results statistically, and found that light and heavy forces produced statistically equal movement of the canine, but heavy force produced more stress in the posterior anchorage segment.

At both force levels, the canine moved more than the molar, but the difference was greater with light forces.

Quinn and Yoshikawa, (1985) support Boester and Johnston's theory that an increase mean stress produces a higher rate of tooth movement but beyond a certain stress level, increasing stress no longer alters the rate of tooth movement.

Contradictory findings have been reported in the orthodontic literature regarding force magnitude and the extent of root resorption. Because of this lack of consensus, the magnitude of orthodontic forces used in root resorption studies has been variable.

2.2.6.2.8.1 Studies investigating force magnitude and root resorption.

Dellinger (1967) applied a 10g, 50g, 100g and 300g intrusive force to the maxillary first premolars of four *Macaca Speciosa* monkeys for 60 days and found that root resorption was directly related to force magnitude. The 10g and 50g forces resulted in moderate root resorption, whereas the 100g force resulted in an increased amount of root resorption and the 300g force resulted in severe resorption.

Stenvik and Mjör (1970) analyzed thirty five premolars that were submitted to intrusive force for a period varying from 4 to 35 days with fixed appliances. The magnitude of intrusive force applied ranged from 35 to 250 grams. An additional untreated 35 teeth served as controls. Histological analysis was carried out after the extractions of the teeth to investigate pulp reactions. The pulp alteration in the experimental teeth could not be directly related to the magnitude of force employed; however according to the authors, there was a tendency towards increased severity with increased force. Resorption of the cementum and dentine was found in 60 percent of the experimental teeth. When the force applied was above 100 grams there was less resorption observed than when the force applied was below 100 grams.

Reitan (1974) examined 72 premolars from individuals 9-16 years old after the application of 25g to 240g of intrusion, extrusion and tipping movements over 10 to 47 days and found that external root resorption was not clearly correlated to force magnitude.

King and Fischlschweiger (1982) applied 50 to 200g of force in rats and found that light forces produced more rapid tooth movements with insignificant cementum cratering whereas intermediate or heavy forces resulted in slower tooth movement and an increased amount of root resorption.

Harry and Sims (1982) used Scanning Electronic Microscopy (SEM) to study the effects of different force magnitudes of intrusive orthodontic forces on the topography of the root surfaces. In this qualitative study, they used a sample of 36 maxillary and mandibular bicuspid teeth indicated for orthodontic extractions. Eighteen teeth were subjected to experimental intrusion and the other half were left as untreated controls. Through a partial banded appliance, intrusive force of 50, 100 and 200 grams were applied over experimental periods of 14, 35 and 70 days. The authors found an increase in the root resorption craters of all experimental teeth when compared to controls. The results showed that the distribution of the root resorption craters was directly related to the amount of stress on the root surface and the rate of resorption development was faster with increasingly applied forces. The amount of root resorption increased with longer periods of intrusive force application

Vardimon et al., (1991) in experiments that lasted 33 – 135 days in monkeys, found that the magnitude of force is the predominant factor when aggravation of root resorption is concerned.

Faltin *et al.,* (1998) and Faltin *et al.,* (2001) applied 50 or 100 cN of continuous intrusive force to 12 upper first premolars, orthodontically indicated for extraction in six individuals for 4 weeks. Examination under the transmission electron microscope demonstrated greater root resorption in those teeth intruded with the heavier force. They concluded that a reduction of continuous force magnitude should be considered to preserve the integrity of the roots.

Casa *et al.,* (2001) used the SEM to analyze 28 upper first premolars that were submitted to 300 or 600cN of continuous torque. All teeth were orthodontically indicated for extraction and derived from 14 patients. The experimental period varied from 1, 2, 3 or 4 weeks. The results demonstrated that teeth that had been moved with a higher magnitude of force showed a higher degree of root resorption in width and depth.

Owman-Moll *et al.,* (1996), examined 32 maxillary premolars after the application of a buccally continuous force. Sixteen teeth were moved with a force equivalent to 50g and the other 16 teeth were moved with a force equivalent to 100g.The right premolar served as the experimental tooth, the left as control. The experimental period took place over 4 and 7 weeks. The results of this study showed that great individual variation existed regarding both the magnitude of tooth movement and the amount of root resorption. The severity of root resorption did not differ significantly when the amount of force applied was doubled to 100g. They concluded that force magnitude was independent of the degree of root resorption and that individual reactions may have more impact than the increase in force or length of the experimental period on both tooth movement and incidence of root resorption.

Furthermore, Owman-Moll *et al.,* (1996) applied a buccally directed continuous force of 50g to the maxillary first premolar on one side and 200g to the maxillary first premolar on the other side in 8 individuals. This experiment took place over seven weeks. Individual variation noticed was once again large. They established that when the applied force was increased to 200g, there was a 50% increase in the rate of tooth movement without any major difference in the frequency and severity of external root resorption.

Chan *et al* (2004) in a recent study analysed the association of the root resorption craters and the magnitude of force applied. The volume of the resorption craters was analysed and quantified in three dimensions using Scanning Electronic Microscopy (SEM) and specially designed software. Twenty-one human first premolars that were subjected to 25 grams or 225 grams of buccal force during a period of 4 weeks were extracted after force application and submitted to analysis. All teeth were orthodontically indicated for extractions. The results show that there was an increase in the amount of root resorption present and the magnitude of force applied. There was a statistically significant difference in the volume of the root resorption craters found in the 225 grams group when compared to the 25 grams group and to the control group.

Darendeliler *et al* (2004) studied the associations of root resorption and the alterations in physical properties, mineral contents and resorption craters in human premolars after the application of light and heavy controlled orthodontic force. They found that there were no significant differences for hardness and elastic modulus between the light and heavy force groups and also no significant effects for different tooth positions. An inter individual variation in the Ca, P and F concentration was observed. The mean volume of the root resorption craters was shown to be greater when heavy force was applied.

2.2.7 Diagnosis of Root Resorption

The diagnosis of root resorption is mainly done though radiographs. Radiographs, however, show limitations in their accuracy. Measurements can only be taken in two dimensions. Buccal and lingual root resorption defects are not visible (Brezniak and Wasserstein, 2002) and digital subtraction can only predict small amounts of apical root loss. (Reukers *et al.,* 1998; Heo *et al* 2001).

CT scans offer distinct advantages over radiographs but due to its cost and radiation exposure protocols for the patients, its use is mainly restricted to research *in vitro.*

2.2.8 Clinical Significance of Root Resorption

The prevalence of root resorption for the great majority of orthodontically treated patients is not of clinical significance and do not appear to increase the risk of tooth loss. (VonderAhe, 1973; Remington *et al.,* 1989)

Vlaskalic *et al.,* (1998) found 6 articles published between the years of 1914 and 1997 that reported cases of apical root resorption posing a problem for either the clinician or patient (Crane, 1926; Miller, 1930; Lussier, 1936; Lyndon Carmon, 1936; Langford and Sims, 1981).

Loss of root length by apical root resorption is less detrimental than an equivalent amount of periodontal attachment loss at the alveolar crest particularly in the initial stages of root resorption (Lupi *et al.,* 1996).

Kalkwarf *et al.,* (1986), using a computer graphics system, calculated the area of periodontal attachment remaining after various lengths of root loss on a central incisor tooth and found that 1mm of apical root loss was equivalent to 5% of periodontal attachment loss. Similarly, a tooth with 5mm of apical root resorption would still have 75% of periodontal attachment remaining.

It has been recommended that after 6 months of treatment, periapical radiographs be taken and where resorption is detected, treatment should be arrested for two to three months with passive arch wires (Levander and Malmgren, 1994,1998,2000).

Ghafari (1994) suggests that where resorption is detected, an additional radiograph be taken in 3 months time.

Levander *et al.,* (1998) suggest 3-monthly radiographic review of blunt and pipette-shaped apices. Where severe resorption is identified, the treatment goals should be reassessed with the patient and a compromised treatment might need to be accepted (Brezniak and Wasserstein, 2002).

Brezniak and Wasserstein (2002) recommend that final records be always taken with compulsory radiographs at the time of fixed appliances removal.

Levander and Malmgren, (2000) recommend follow-up radiographic examinations until further root resorption is no longer evident for teeth with severe resorption.

Roberts (2000) suggests that retaining teeth with fixed appliances should be done with caution. Occlusal trauma of the fixed teeth or segments might lead to extreme resorption.

Remington *et al.* (1989) looked at 100 cases that experienced root resorption and found that the worst outcome was hyper mobility, which occurred in only 2 cases.

Vlaskalic *et al.* (1998) stated that the sequelae of orthodontically related resorption does not pose a long-term threat to the patient.

Levander and Malmgren (2000) conducted a long-term follow-up of maxillary incisors with severe apical root resorption from 5 to 15 years after orthodontic treatment. They found a significant correlation between tooth mobility, total root length and intra-alveolar root length. They concluded that there is a risk of tooth mobility in a maxillary incisor that undergoes severe root resorption during orthodontic treatment if the remaining total root length is less than or equal to 9mm. They reported a decreased risk if the remaining root length is greater than 9mm and recommend follow-up of teeth with severe orthodontically induced root resorption.

2.3 X- Ray Microtomography

2.3.1 History and Development

The discovery of X-rays, more than one hundred years ago, led to a revolution in diagnostic medicine, making it possible to see inside the body non-invasively. (Davis Wong, 1996; Stock, 1999)

In 1963, Cormack demonstrated the feasibility of using x-rays absorptivity within a cross section of an object. In 1973, Hounsfield developed a commercially available computed tomography system for medical imaging. This brought a further significant step forward in diagnostic medicine since it allowed images of internal features to be made based directly on their X- ray attenuation coefficients. (Davis and Wong ; 1996 Stock,1999)

In Hounsfield's original design, the XMT arrangement consisted of an X- ray source, a pinhole collimator and a single detector (first generation system).

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-rays projections through the slice at various angles around an axis perpendicular to the slice. (Davis and Wong, 1996)

X-ray micro tomography (XMT) is a miniaturized version of computerized tomography with resolution in the order of micrometers approaching that of optical microscopy. It allows imaging of the interior microstructure of the materials non-destructively and non invasively. The same object can be rescanned after appraisal or the same sample can be assessed multiple times during testing (Davis and Wong, 1996). Computed tomography provides an accurate map of the variation of X-ray absorption within an object, regardless of whether there is a well defined substructure of different phases or slowly varying density gradients (Stock, 1999).

Before computed tomography, laminography was used. It involved the translation of the patient or object to be imaged, together with the detection medium (which was the film or another two-dimension detector) in such a way that only one narrow slice parallel to the translation plane remained in focus. Computed tomography became possible with the advance of digital computers. (Stock, 1999)

Due to the increase in demand for the use in new applications, resolution of even smaller features was required and became the goal of computed tomography experts. Instead of resolving features with dimensions that were just smaller than millimetres, one was able to image features of the scale of 10 micrometers allowing the many microstructure features of engineering materials to be studied non- destructively and without a need for sample preparation. This size scale is also important in biological structure of materials such as calcified tissue (Davis and Wong, 1996).

In 1982, Elliot and Dover developed the first X-ray microtomography (XMT) machine and produced an image of hard tissue specimens with a resolution of 12 μm. They imaged the shell of *biomphalaria glabrata* (snail). This happened about ten years after the invention of the CT scanner and the system used was a scaled down version of the conventional medical scanner and could be used for the study of specimens measuring as little as 1 mm across. They used Hounsefield's principles with a 15μm diameter X- ray beam, mounting the specimen on a stage, which moves it through the beam and also rotates it axially.

In medical tomography it is possible to obtain a resolution of a fraction of a millimeter, but whatever technological advances are made in detector design, it will not be possible to improve resolution much further than this because of X-ray dosage limitations. To improve the resolution by a factor of two the exposure must be increased by a factor of 16, with a ten thousand-fold increase in exposure required for only a ten-fold improvement in resolution. With X ray microtomography, since the specimen size is smaller, lower X- rays energies are used and the measured attenuation coefficients are higher. Thus, the signal (attenuation coefficient) to noise ratio, a measure of image quality, can be maintained with a one thousand-fold increase in exposure for a tenfold improvement in resolution (Davis and Wong, 1996).

Apart from the scale, XMT systems differ from medical CT scanners in one fundamental way; instead of the X-ray source moving around the subject, it is the specimen that moves whilst the x- ray source and detector are stationary.

Current technologies limit the use of X-ray microtomography mainly to small, in vitro specimens. However, Kinney *et al.* (1995) showed the use of in *vivo* XMT for studying changes in bone associated with ovariectomy in rats.

The applications of micro tomography are numerous and include fatigue analysis, performance prediction of materials and the study of calcified tissues, like bone and teeth, among others (Mercer and Anderson, 1996).

It is not the intention of this review to explore the mathematics and physical fundamentals of X- ray computed microtomography.

2.3.2 X- ray microtomography in calcified tissues

Elliot *et al* (1989) reported the measured linear attenuation coefficient of enamel and dentin in a human premolar using X- ray microtomography.

Gao *et al* (1993), quantitatively showed how much mineral was lost after demineralization in tooth the tooth rods and how much mineral was regained after remineralization.

Fearne (1994) used X- ray microtomography to determine the mineral concentration in enamel of deciduous teeth from low birth weight children.

Kinney *et al.* (1994) used X-ray microscopy to quantitatively map mineral concentrations in carious dentin. Their data analysis indicated that X- ray tomography microscopy is able to image variations in the mineral concentration in carious dentin with a spatial resolution that is adequate to detect calcified and enlarged tubuli spaces in the lesion.

Nielsen (1995) used XMT to study the root canal of endodontically prepared human maxillary molars.

Mercer *et al* (1996) developed a pilot study to analyse the effects of application of a continuous wave carbon-dioxide laser on dental enamel using X ray micro tomography (XMT). This paper established that XMT was of particular value in the study of the cracking induced by lasers and the authors suggested that the technology should be applied in several areas of dental research.

Dowker *et al* (1997), showed the application of a micro tomography system to the study of root canal morphologic characteristics and changes in the course of root canal treatment in extracted teeth. Using a special software package, cross section slices of the scanned teeth were obtained for observations. The resolution obtained by the authors on this study was of approximately 40 microns.

Wong *et al* (2000) used X-ray microtomography to study the mineral concentrations in sequential slices of enamel of rat mandibular incisors.

Groth *et al.* (2001) used X- ray micro tomography to compare the subsurface enamel beneath laser and acid conditioned, laser-only etched and acid-only etched surfaces. They found that laser and acid conditioning increased the etching depth, but did not induce significant damage in the enamel subsurface. When analysis of the laser-only etched enamel subsurface was carried out, it revealed a small reduction in mineral concentration suggesting an increase in porosity, allowing greater penetration of acid and resulting in an increased acid-etch depth.

Stock *et al* (2002) used X-ray absorption micro tomography and X-ray diffraction mapping, to study teeth of the sea urchin *Lytechinus variegatus*. They found that the technology of the X-ray micro CT and micro diffraction data used in conjunction with protein distribution data would help future researchers to understand the properties of various bio composites and their mechanical functions.

Stock *et al* (2003) conducted a study to investigate bone regeneration in the forelimbs of mature *Notophthalmus viridescens*. Using non-invasive X-ray micro tomography, they imaged the regenerating limbs from 37 to 85 days and also imaged contralateral controls to investigate the onset of mineralization of specific bones, the level of mineral present and the lengths of different bones.

Furthermore, Stock *et al* (2003) published a study that reported the results of what the authors called a novel approach. The use of X-ray micro CT, for quantifying stereom structures applied to ossicles of the sea urchin *Lytechinus variegatus.*

Stock *et al,* once more in 2003, used two noninvasive synchrotron x-ray techniques: x-ray absorption microtomography (micro CT) and x-ray diffraction mapping to map the spatial distribution of mineral at the 1.3 micron level in a millimeter-sized fragment of a mature portion of the keel in the sea urchin teeth.

Mercer *et al* (2003) demonstrated the progressive growth of enamel and dentin craters after the sequential application of an Er: YAG laser using X-ray micro tomography. Laser craters were created in blocks cut from human enamel and / or dentin. Micro Ct was used to visualize and quantify of the effects. The measurements were compared with previous studies that used different protocols. The authors concluded that 3D X-ray micro tomography was proven to be a useful tool for quantitative measurements in dental research.

For reason of high radiation dose, it is unlikely that X-ray microtomography (XMT) will ever be carried out *in vivo* in humans. Nevertheless, the non-destructive nature of the technique allows a variety of experiments to be conducted to investigate structural and compositional characteristics of calcified tissues.

To the present date there are no studies published in the orthodontic literature investigating root resorption using x-ray microtomography technology.

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4. Manuscript

Physical properties of root cementum: Part 8. Volumetric analysis of root resorption craters after application of controlled intrusive light and heavy orthodontic forces: A micro CT Scan study.

This manuscript is to be submitted to the American Journal of Orthodontics and Dentofacial Orthopedics.

Physical properties of root cementum: Part 8. Volumetric analysis of root resorption craters after application of controlled intrusive light and heavy orthodontic forces: A micro CT Scan study.

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ABSTRACT

Intrusion is defined as one of the critical types of orthodontic tooth movement in relation to external root resorption. The aim of this prospective randomised clinical trial was to quantify, three-dimensionally, the amount of root resorption present when controlled light and heavy intrusive force magnitudes were applied to human premolars and to establish the sites where root resorption is more prevalent. Fifty-four teeth orthodontically indicated for extractions were obtained from 27 patients (left and right first maxillary premolars from each patient) and were intruded for a period of 28 days using buccal and palatal β-titanium molybdenum alloy (TMA®) 0.017" x 0.025" cantilever springs.

The patients were randomly divided into three groups and received different levels of force: Group 1, heavy force (225 g) on one side and control force (0 g) on the contra-lateral side; Group 2, light force (25 g) on one side and control force (0 g) on the contra-lateral side and Group 3, light force (25 g) on one side and heavy force (225 g) on the contra-lateral side. After the experimental period, the teeth were extracted under strict protocol to avoid root surface damage and analysed using a micro CT scan X-ray system (SkyScan-1072, Belgium) and specially designed software for direct volumetric measurements (CHULL2D).

The volume of root resorption craters after intrusion was found to be directly proportional to the magnitude of intrusive force applied. The results showed that the control group have less and smaller root resorption craters, the light force group have more and larger root resorption craters than the control group, and the heavy force group have the most and the largest root resorption craters of all groups. A trend of increase in the volume of the root resorption craters was observed from control to light to heavy group and these differences were statistically significant. The mean

volume of the resorption craters in the light and heavy force groups was 2 and 4 times greater than the control group, respectively. The mesial and distal surfaces presented the greatest resorption volume with no statistically significant difference between the two surfaces.

Key words: Root resorption, premolars, intrusion, light and heavy orthodontic forces, X-ray micro tomography (XMT), three-dimensional analysis, volumetric measurements.

INTRODUCTION

Root resorption can be described as an inevitable pathological outcome of orthodontic tooth movement. Although the orthodontic literature presents many studies that investigated the external root resorption phenomenon, it still remains a challenge for researchers to identify all the factors that interfere with the biological system and are responsible for causing resorption after the application of orthodontic forces with different magnitudes.

Orthodontic intrusion is described as one of the worst types of orthodontic tooth movements in relation to external root resorption[1].

Previous authors have investigated root topography after controlled intrusive orthodontic movement, but did not quantify the amount of root resorption present in three dimensions[1,2].

Quantitative analysis can be accomplished using radiographs, but two-dimensional studies are limited to measuring only small amounts of root apex loss[3,4]. In addition, magnification errors might lead to under or over-estimation of the amount of root resorption present.

Volumetric analysis of root resorption craters using Scanning Electronic Microscopy and specially designed software was shown to be effective in obtaining a three-dimensional quantitative analysis of root resorption craters[5]. This study demonstrated a direct association between the volume of the root resorption craters and the magnitude of buccal force applied to the teeth during the experimental period. Compression of periodontal ligament associated with intrusion results in root resorption[6]. Although all types of tooth movement can cause resorption, intrusion might produce a significant decrease in root length and causes a great amount of relative stress at the apex[7]. To date, there has been no attempt to quantify in three-dimensions the amount of root resorption present after controlled orthodontic intrusion.

The micro CT scan system used in this study (SkyScan-1072, Belgium) is a desktop X-ray system designed for non-destructive and non-invasive three-dimensional imaging of the microstructure of the teeth with high spatial resolution. It allows the reconstruction of the teeth in three dimensions and is able to reproduce 5-micron slices.

The aims of this study were to present a newly developed method for accurate quantitative analysis of root resorption craters, to establish the effects of different intrusive force magnitudes on the volume of the root resorption craters and to identify the surfaces where root resorption is more prevalent after controlled orthodontic intrusion of human premolars. The technique described below eliminates some of the complexities that are present when three-dimensional analysis is carried out using Scanning Electron Microscopy such as specimen preparation and graphical reconstruction of the resorption craters[5].

MATERIALS AND METHODS

Fifty-four maxillary first premolars were collected bilaterally from 27 patients who required extractions for orthodontic purposes (Ethical approval – Project 5/98 of Human Ethics Review Committee - CSHAS). Patients selected (12 males and 15 females, mean age of 15.6 (range 11.9 – 19.3 years) were ready to start comprehensive orthodontic treatment. Due to the differences in the maxillary and mandibular bone density and root anatomy, only maxillary upper premolars were used for this study.

Patients were selected following strict criteria described previously[8]. All subjects following selection completed a written informed consent and a questionnaire regarding fluoride intake, birthplace and previous living places. The subjects were then randomly divided into 3 groups (9 individuals and 18 teeth for each group): Group 1 for light, controlled orthodontic force of approximately 25 g on one side and control force (0 g) on the contra-lateral side. Group 2 for heavy controlled orthodontic force on one side of approximately 225g and control force (0 g) on the contra-lateral side and Group 3 for light controlled orthodontic force of approximately 25g on one side and heavy controlled orthodontic force of approximately 225g on the contra-lateral side. The right or left premolars were randomly chosen for heavy, light or control force application.

Every patient had an alginate impression taken of the maxillary arch. The impressions were poured in plaster for posterior appliance construction.

The appliance consisted of 0.022-inch slot Victory brackets (3M – Unitek, USA®) that were bonded on the plaster model on the buccal and palatal surfaces of the maxillary first premolars on both right and left sides for posterior an intra-oral indirect bonding procedure and a transpalatal arch linked by two acrylic occlusal cover caps to be cemented on the maxillary first molars. The purpose of this design was to avoid molar movement or occlusal interferences on the premolar teeth during the experimental period. Each acrylic occlusal cover cap had 0.022-inch slot SPEED brackets (Strite Industries, ON, CA) added on both buccal and palatal surfaces. Two β-titanium molybdenum alloy (TMA®) 0.017" x 0.025" cantilever springs were then constructed on the model on the buccal and palatal surfaces and engaged in the first molar brackets (Figure 1 A, B). These springs were used to deliver the required apical force and were calibrated to the nearest gram with a strain gauge (Dentaurum, Germany). Once the subject was assigned to a specific force group, the transpalatal bar was cemented using Multi Cure Glass Ionomer cement (3M – Unitek, USA®) and the brackets on the premolars bonded indirectly. The preformed cantilever springs were transferred intra-orally and recalibrated according to the appropriate force required. The contra-lateral first premolar served as a control in groups 1 and 2. Premolars and molars were also bonded on the contra-lateral side (control side) but no springs were placed. (Figure 2 A, B).

In group 3, both the right and left maxillary first premolars were used as experimental teeth and apically activated springs were issued for both sides. Heavy force (225 g) was applied on one side and light force (25 g) on the contra-lateral side.

Several prototypes were tested before the appliance design chosen for this study was finalized. In an attempt to minimize tipping during intrusion of the teeth, the same magnitude of force was applied to all experimental premolars on both buccal and palatal surfaces. The force magnitude was divided in half for each cantilever spring, so that the same force level was applied on the buccal and palatal surfaces totaling 25g for the light force group and 225g for the heavy force group.

There was neither buccal nor lingual activation of the springs, as this would encourage bracket disengagement. Thus, concern expressed in a previous study[5] regarding spring ligation was not relevant to this design. All springs were bent extra-orally to be transversally passive in the brackets in order to minimize tipping forces during intrusion. After activation, the springs were re-tested to assure that no torquing forces were applied. Moreover, the force was divided equally between the buccal and palatal springs for the same reason. The springs were double ligated with ligature wires and an elastomeric ring to assure full bracket engagement and continuous force application.

The premolars were relieved from function during the experimental period via the placement of acrylic occlusal stops on the molars. These stops also prevented distortion of the springs.

The duration of the experiment was 28 days. During this period, no spring was re-activated. One operator treated all of the patients. Initially 32 patients were selected. Two subjects took part in a pilot study for the test of spring design and three subjects could not take part in this study due to breakage of the springs during the experimental period. After the experimental period, the teeth were extracted by two oral surgeons, who followed a previously established protocol to avoid damage to the root cementum[5]. After extractions, the teeth were placed in individually marked containers of de-ionized water (Milli-Q®), which has been previously found to be an appropriate storage medium[8]. An ultrasonic bath was used for a period of ten minutes to remove all traces of residual periodontal ligament (PDL) and soft tissue fragments. All visible signs of PDL remaining were removed with damp gauze. The teeth were disinfected in 70% alcohol for 30 minutes and re-stored in the individual containers of de-ionized water (Milli-Q®). Prior to analysis, the teeth were bench dried for a minimum of 48 hours. Subsequent analysis of the teeth was carried out using a desktop micro CT X-Ray system (SkyScan-1072, Belgium). No further specimen preparation was necessary preceding analysis. Customized software (CHULL2D) was developed for direct volumetric measurements of the craters at the Electron Microscopic Unit – University of Sydney.

MICRO CT SCAN

X-ray microtomography (XMT) is a variant of a medical CT scan system that allows imaging of the interior microstructure of materials to be obtained non-destructively and with high spatial resolution[9]. The software enables the reconstruction of the complete internal microstructure of the teeth complete internal microstructure of the teeth and it is able to reproduce fully three-dimensional data sets with isotropic sample spacing down to approximately 5-microns. (SkyScan-1072, Belgium).

Imaging acquisition

The teeth were scanned one at a time. This procedure took approximately 60 minutes per tooth. During cone beam acquisition the teeth rotated over 180 degrees. At each position the shadow image or X-ray absorption radiograph was acquired. All teeth were scanned 2 to 3mm coronal to the cemental enamel junction (y position) with a magnification augment equivalent to sixteen times (17.08 μm pixel size) using a rotation step of 0.45° and exposure time of 1.904 seconds. A total of 420 X-ray absorption radiograph images were acquired for each tooth during this first step and they were saved as 16 bit TIFF (Tagged Image File Format) files.

After acquisition was completed, axial slice-by-slice reconstruction was undertaken using a specific software implementation of the Feldkamp cone-beam algorithm[10] (*Cone beam reconstruction 2.13 – Skyscan –1072, Belgium*).

The 16-bit TIFF shadow images generated a raw reconstructed cross-section data by using the reconstruction algorithm. The individual axial slices were generated as 1024x1024 pixel bitmap (BMP) images having an 8-bit gray-scale dynamic range. The gray-values in each dataset were calibrated so that the 8-bit range fully mapped the variation between the pixels with maximum X-ray attenuation (i.e. the most opaque) and those with minimum X-ray attenuation (i.e. transparent). A total of one thousand two-dimensional axial images for each tooth were generated.

Three-Dimensional Image Visualization

The 1.1 version of *VG Studio Max 1.1 software (Volume Graphics GmbH, Germany)* was used for the three-dimensional reconstruction of the tooth images. It allowed visualization in three dimensions of all aspects of the scanned tooth (Figures 3A, 3B). The saved two-dimensional BMP files were acquired and reconstructed in 3 dimensions. The four surfaces of the teeth (buccal, lingual, mesial and distal) were analysed. Each one of these surfaces was divided into thirds (cervical, middle and apical). All the root resorption craters present were acquired and saved separately according to its location on the root surface. They were saved as a different group of BMP images (Figure 4 A, 4B).

Volumetric measurements of the root resorption craters

All craters were measured individually and then, the sum for the root resorption volume for each tooth and for each surface was obtained. The volumetric measurement was obtained using convex hull software (CHULL2D) that was developed specifically for this project at the Electron Microscope Unit at the University of Sydney.

After the three dimension reconstruction of the craters, the images were stored are as cross sections of the total sample.

The volume measurement software (CHULL2D) is based on the application of a two-dimensional convex hull[11] that is generated for each axial slice of the crater data set. The algorithm assumes that the surface of the tooth is convex in nature, therefore when a crater present, a break in the convexity is present and a volume is possible to be detected. The convex hull algorithm (CHULL2D) reconstructs the loss of convexity by estimating a new line of closure that effectively connects the two points at the edge of the break, hence creating a closed sub-volume for each axial slice of the crater. The total crater volume is calculated by summation of the sub-volumes for these closed cross sections.

RESULTS

The three-dimensional images of the teeth were saved as permanent files. The method by which the craters were acquired was recorded with respect to the x, y and z-axis. Therefore, repetitions of tests were not necessary.

Univariate Analysis of variance (ANOVA) was performed by Statistical Package for Social Sciences (SPSS for Windows, version 12, SPSS Inc, Chicago, Ill). During statistical analysis, the raw data were transformed for the residual plots to conform to normality. The cube root of the volumetric readings was used to create a model for statistical analysis. Essentially, this replaces each volume by the radius of an equivalent hemispherical crater. This approach was also used in a previous study[5]. The factors used in the model were: subject (random), force, surface and thirds (fixed).

The data from all groups were analyzed using an ANOVA model with all main effects and two factor interactions. Overall, force and surface showed a significant main effect. Height was marginally significant but subject was not. The surface by subject interaction was significant, but was based on large numbers of degree of freedom and hence is not important practically. Table 1 shows the significance of the factors and interactions.

The three force levels are significantly different from each other, even with a Bonferroni adjustment. Heavy force to light force $p < 001$, light force to control $p = 0.027$ and heavy force to control $p < .001$. An increase in the volume of the root resorption craters was observed from control to light to heavy group (Table 2) (Figure 5).

The results consistently showed that the control group have less and smaller root resorption craters, the light force group have more and larger root resorption craters than the control group, and the heavy force group have the most and the largest root resorption craters of all the groups.

The analysis showed that the mean cube root volume of the resorption craters in the light and heavy force groups was about 2 and 4 fold times greater than the control group respectively.

The analysis also showed significant differences in the mean cube root volume of the resorption craters between tooth surfaces. (Table 3)

The mesial and distal surfaces are significantly different to the palatal and buccal surfaces ($p<0.001$ in each case), but there is no significant difference within the groups.

There was no significant difference in the volume of root resorption craters between the three thirds of the root. However, there was a trend showing that the apical third had the greater resorption volume. (Table 4)

DISCUSSION

It is recognized in the orthodontic literature that long-term intrusion will cause external root resorption and a decrease in root length[1,12,13]. Root length loss was not considered to be significant during this study due to the short experimental period. However, the loss of root length in the present study samples will be the subject of future analysis.

Previous clinical studies that have attempted to investigate root resorption after intrusion[1,2,12-14] did not demonstrate a three-dimensional quantitative analysis of the amount of root resorption observed. Besides, there were substantive methodological differences amongst these studies making their results difficult to compare. The present study evaluates the effects of different force magnitudes during short term controlled intrusion and also analyses quantitatively, all the surfaces of the tooth root after intrusion.

Quantitative analysis of root resorption craters using radiographs or histological analysis has been proven to be inaccurate, difficult to reproduce and technique sensitive[15]. However, when three-dimensional volumetric measurements of root resorption craters were compared to two-dimensional surface area measurements of the same sample after 28 days of force application, a significant correlation between the two measurements was found[16]. A longer experimental time comparing the two techniques might be needed before any definite conclusions are reached.

The mean cube root volume of the resorption craters in the light and heavy force groups was found to be about 2 and 4 fold times greater than the control group respectively. There was a statistically significant linear increase in the volume of the root resorption craters from control to light and from light to heavy force groups.

The heavy force group had a significantly greater volume of root resorption craters than control and light force groups agreeing with previous animal[17,18] and human studies[1,2,5]. Reitan[19,20] has proposed the use of light forces during orthodontic treatment in order to increase the cellular activity in the surrounding tissues and reduce the risk of root resorption. Qualitative studies on intrusion demonstrated similar results. Harry and Sims[1] showed that the distribution of the root resorption craters is directly related to the amount of stress on the root surface. Faltin *et al.*[2,21], showed that greater root resorption is observed in teeth intruded with heavy force. The present findings also conform to the results of a previous quantitative root resorption study that compared different force magnitudes[5].

Contradictory findings have been reported in the orthodontic literature regarding force magnitude and the extent of root resorption. Some studies state that an increase in force magnitude is not directly proportional to an increase in root resorption[12,22,23]. These studies, however, were histological studies and did not quantify the amount of root resorption present in three dimensions. Different methodological designs might be responsible for the lack of consensus regarding force magnitude and root resorption. The magnitude of orthodontic forces used in root resorption studies has been variable[12,24].

Schwartz[25] suggested in his pressure-tension theory that the optimal force level for tooth movement should be between 7 and 26 grams per square centimetre. He also stated that, when force exceeded this threshold, root resorption would occur. When pressure decreases to below this threshold, root resorption ceases [22].

Storey and Smith[26] introduced the optimal force theory proposing that a range of pressure between 150 to 200 g would produce the maximum rate of tooth movement for distalization of maxillary canines in man. They proposed that if the force was increased beyond this optimum range, hyalinisation and undermining resorption could happen. Therefore, the 25g force was selected as a relatively light force level and a nine-fold greater force of 225 g was considered as a relatively heavy force level. The force range on this study was also selected to conform to previous resorption studies that have been developed at the University of Sydney. This allows methodological designs to be similar and results to be judged against in the future[5].

It has been demonstrated that β Titanium Molybdenum Alloy wires (TMA) performed well when a long range of activation was required with almost insignificant force decay over a prescribed active range[27]. Force decay was not significant and the spring design for this study did not require reactivation during the experimental period. This also replicated precisely the clinical picture where there are no reactivations of the orthodontic appliances in between appointments.

To move a tooth through bone, compression of the periodontal ligament is necessary making all moved teeth vulnerable to external root resorption. However, individual susceptibility can be so erratic, that it is not viable to predict which patient will experience root resorption and how severe it will be under the same orthodontic treatment protocols. Although strict criteria were used for patient selection[8] , ethnic background was not taken into account. Patients who took part on this study belonged to nine different racial backgrounds. Sameshima and Sinclair[28,29], after examining records from 868 patients found that Hispanic patients were more susceptible to root resorption than Asians or Caucasians. This might be an important point for future directions in root resorption research; however in practical terms it would very inappropriate to limit a study to a single background subject sample in the multicultural patient range that attend our university clinic. Despite this fact, the design of this experiment (comparing controls within the same patient) allowed in some way for definite conclusions to be established. Two out of the 27 subjects that were used in this study were from Hispanic background. The amount of root resorption found for these subjects did not differ to the average amount of root resorption found for the other subjects on the same force group.

Scanning electron microscopic studies were able to detect root resorption cavities after a relativelly short period of time of force application[1,30,31]. Despite the fact that the experimental period was short during the present experiment, it does reflect the true clinical routine for orthodontic treatment. Significant resorption was observed during the experimental period of 28 days. The extent of the experimental period allowed for the springs to be tolerated by the patients avoiding breakages and minimizing force decay.

The crater images acquired in this study were not graphical simulations. They were the exact tooth image that was simply magnified. The micro-tomography images allowed observation of the morphology and particular aspects of the tooth anatomy.

Morphological perforations or defects that are a common occurrence on the tooth root were able to be clearly distinguished from root resorption craters and were not acquired as craters. It was possible to explore the morphology of root resorption craters that were perforated and communicated with the root canal. Several craters in the heavy force groups often presented micro communications with the root canal and several others were located adjacent to accessory canal foramina. None of these observations were possible from previous studies using scanning electron microscopy[15]. The major limitation associated with the volume estimation software used in this study relates to the way in which the craters are closed by the convex hull algorithm. In general a convex hull creates a surface closure that is a local minima in terms of its surface area. This effectively creates a flat closure that approximates the original tooth surface to a greater or lesser extent depending on the curvature of the tooth surface at the site of the crater. Where tooth surface curvature is high (ie towards the apex of the root) and the crater large in lateral extent a flat closure will be more likely to under estimate the crater volume. This small error is mitigated however by the fact that this method of crater volume measurement is based on a direct imaging of the tooth as a fully 3D object. Hence the error in volume estimate was considered to be negligible.

Root resorption was found on the four surfaces of the teeth. However the mesial and distal surfaces presented a significant increase in the volume of root resorption craters when compared to the buccal and lingual surfaces. This might be explained by the fact that, despite the effort made to achieve pure intrusion and minimize buccal lingual tipping by the placement of springs on both palatal and buccal surfaces, mesio-distal tipping might have occurred resulting in pressure on the teeth-bone interface on these surfaces. The teeth were in malocclusion and crowded positions before the experimental period. In spite of the best attempts, intrusive force direction might not have been, in its totality, applied perpendicular to the long axis of the tooth. Also, the crowding of the surrounding teeth might have influenced the amount of intrusion obtained. The apical thirds of all surfaces presented the greater volume of root resorption craters, which conforms with the results from a previous study[7] which stated that stress after intrusion is concentrated mainly at the apex. Harry and Sims[1] observed craters present on the bucco-cervical third and the linguo-apical third after intrusion, however intrusive force was applied on the buccal surface only and away from the centre of resistance of the tooth allowing bucco-lingual tipping. Faltin *et al*[2] also observed greater resorption in the apical region followed by the middle third of the root.

All patients had a panoramic radiograph taken prior to treatment but periapical radiographs were not requested for research purposes for obvious ethical rationale. Panoramic radiographs were shown to be inaccurate methods of root resorption detection[32]. Pre-existing root resorption or root shortening was not visible three dimensionally before the experimental period. Despite the fact that the teeth were carefully selected, making sure the apexification was complete and avoiding any predisposition to root resorption, resorption craters were also found on the control teeth. It is clear that, for most of our patients, orthodontic biomechanics are not the sole factor that may interfere with the biological system and cause root resorption.

CONCLUSIONS

Root resorption studies are now being developed in a new technological era where three-dimensional measurements with a high degree of accuracy are possible to be obtained. Methods used in the past for root resorption assessment were somewhat restricted and inaccurate. Quantitative scanning electron microscopic analysis adds complexities to the methodological designs that are eliminated with the use of micro-tomography. Besides, X-ray micro-tomography is accurate in differentiating important anatomical features of the tooth root.

Based on our results we can come to the following conclusions:

- Root resorption was found to be directly proportional to the magnitude of the intrusive force applied. There was a statistically significant increase in the volume of the root resorption craters from control to light and from light to heavy force groups.
- The mean cube root volume of the resorption craters in the light and heavy force groups was about 2 and 4 fold greater than the control group respectively.
- The mesio-apical and disto-apical surfaces demonstrated significantly more resorption volume than the other regions of the tooth roots with no statistically significant difference between themselves.
- Despite the fact that force magnitude and orthodontic biomechanics are not the sole factor that may lead to the occurrence of external root resorption, the use of

heavy forces during intrusion should be undertaken cautiously, especially if this procedure will be carried out over a long period of time.

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LIST OF FIGURES

Figure 1: (*A*) Appliance design for Groups 1 and 2 constructed on a plaster model. (*B*) Appliance design for Group 3 constructed on a plaster model.

Figure 2: (*A*) Intra-oral view of experimental side (Note the disocclusion of the premolars due to acrylic occlusal covers on the maxillary first molars). (*B*) Control side showing the contra-lateral premolar (Groups 1 and 2).

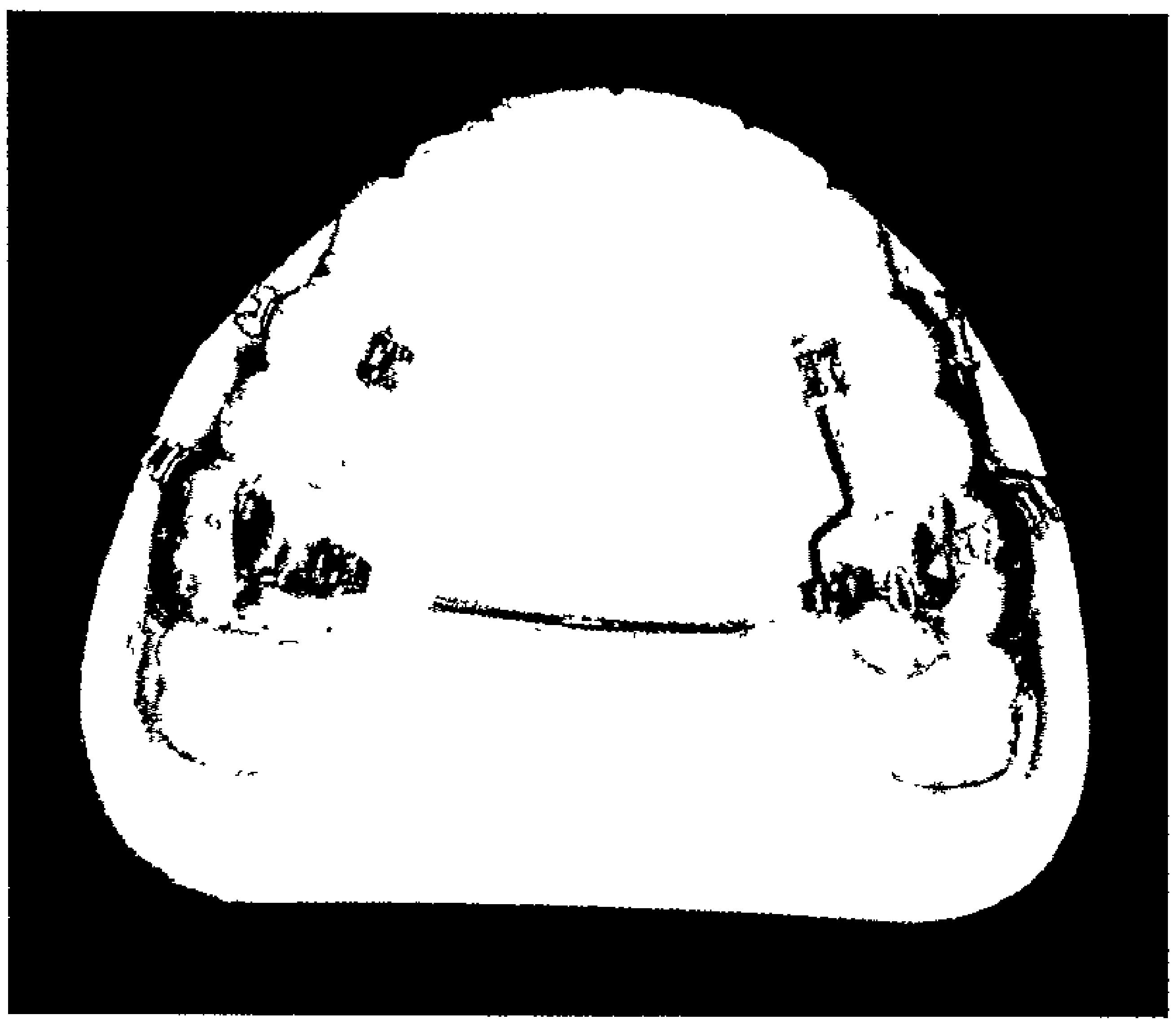
Figure 3: (*A*) Micro Ct Image of a maxillary first premolar showing an external root resorption crater on the apical third of the palatal surface. (*B*) Root resorption crater shown in detail.

Figure 4: (*A*) Root resorption craters present on the middle third of the root (mesial surfaceOclose to the furcation area of a maxillary premolar. (*B*) Root resorption crater shown in detail with magnification equivalent of 42 times.

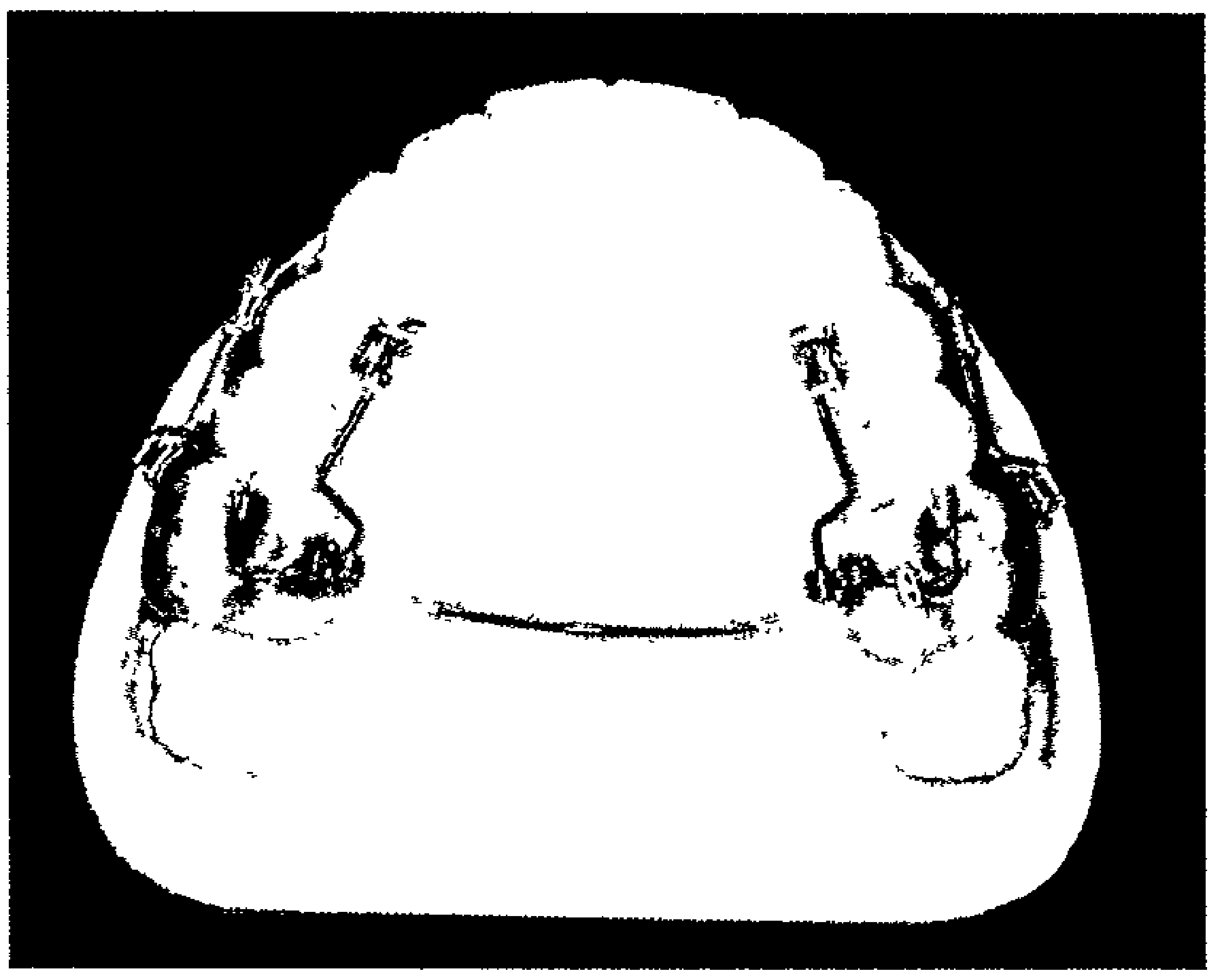
Figure 5: Boxplots of the cube root total volume for different force levels.

LIST OF TABLES

FIGURES



A



B

Figure 1: (*A*) Appliance design for Groups1 and 2 constructed on a plaster model. (*B*) Appliance design for Group 3 constructed on a plaster model.

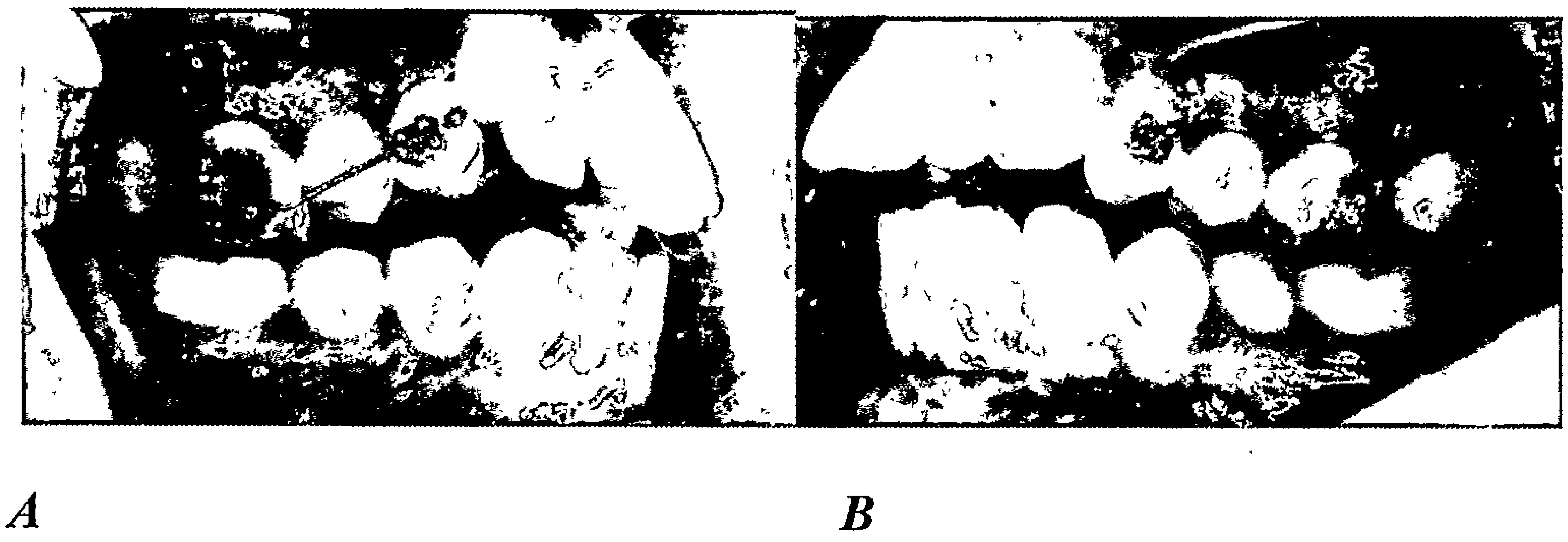
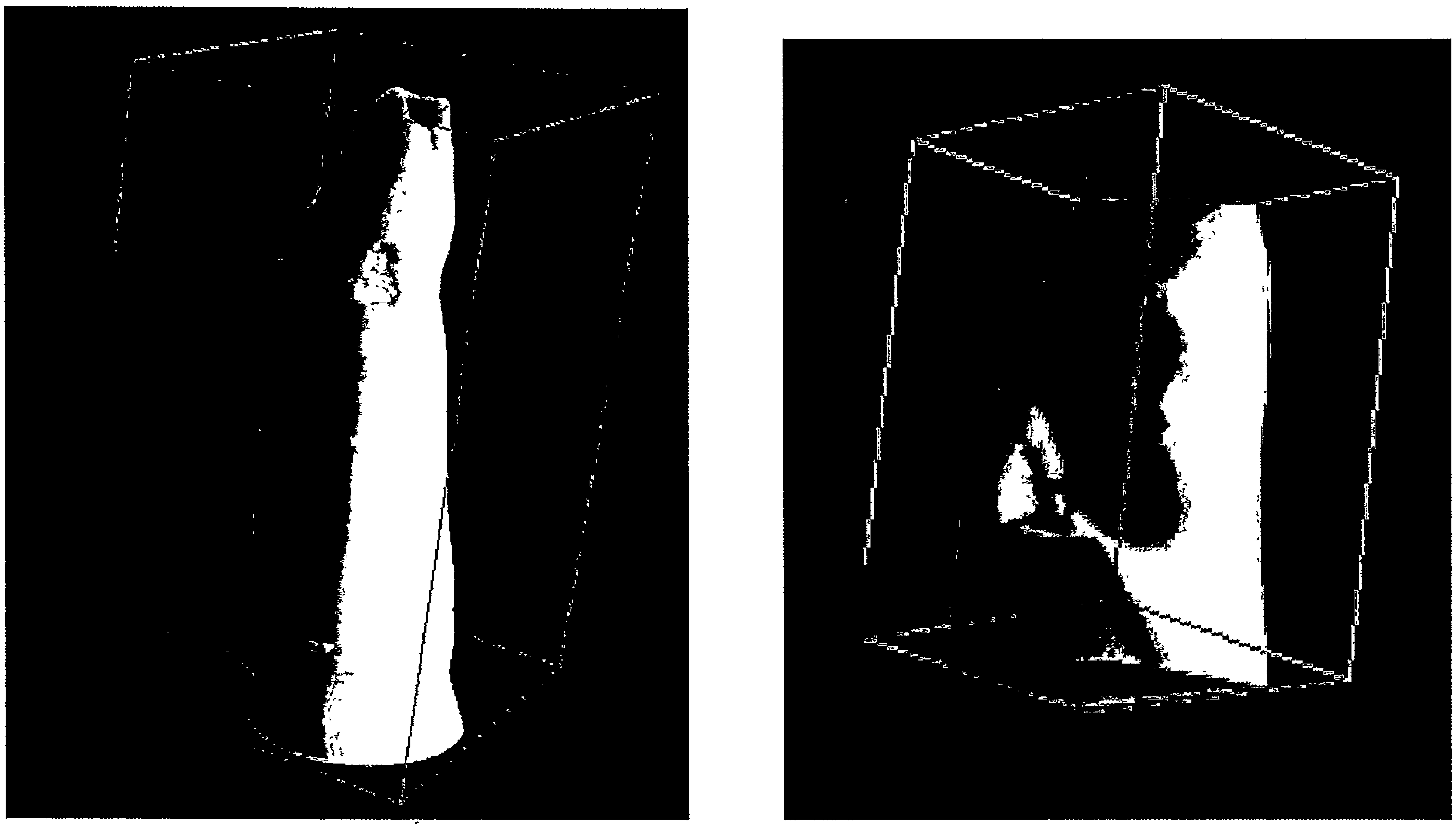
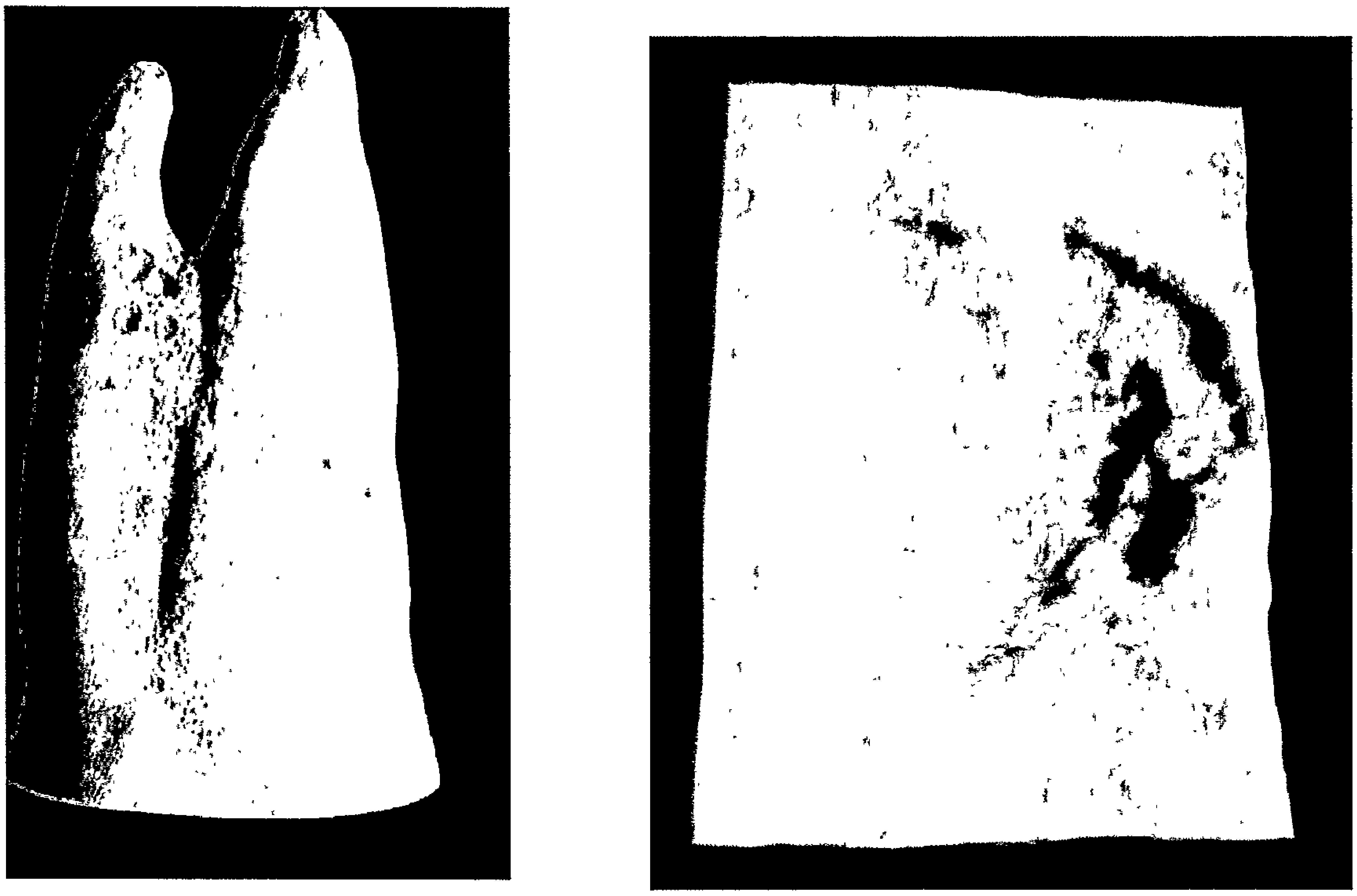


Figure 2: (*A*) Intra oral view of experimental side (Note the disocclusion of the premolars due to acrylic occlusal coverage on the maxillary first molars). (*B*) Control showing the contra-lateral premolar (Groups 1 and 2).



A B

Figure 3: (*A*) Micro Ct Image of a maxillary first premolar showing external root resorption crater on the apical third of the lingual surface. (*B*) Root resorption crater shown in detail.



A B

Figure 4: (*A*) Root resorption craters present on the middle third of the root (mesial surface) close to the furcation area of an upper maxillary premolar. (*B*) Root resorption crater shown in detail with magnification equivalent of 42 times.

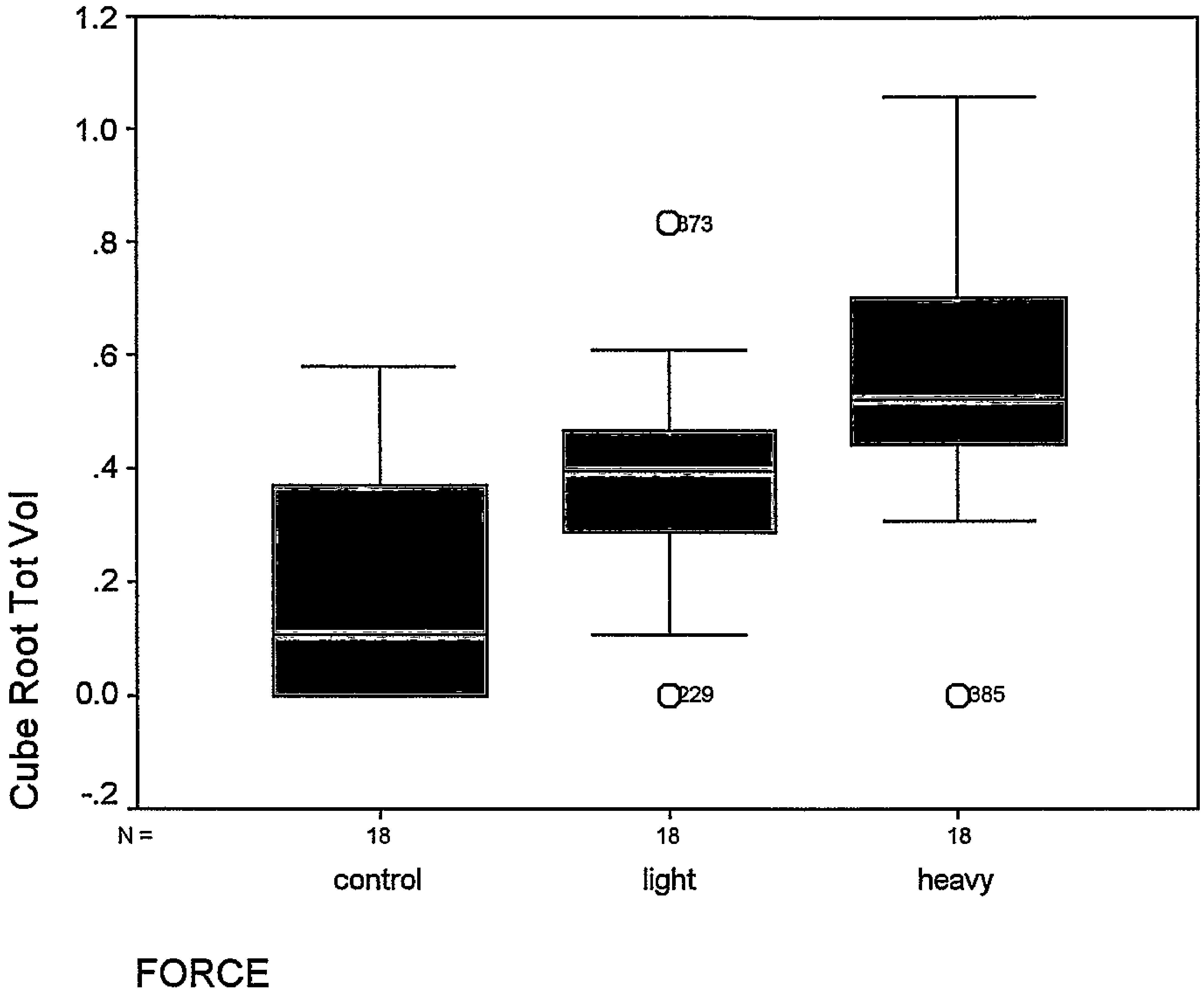


Figure 5:Boxplots of Cube root of the total volume for different force levels

TABLES

Table 1: ANOVA results for cube root Volume

SOURCE	d f	F	Sig.
SUBJECT	26, 21.3	1.09	.42
FORCE	2, 25	14.98	.000
SURFACE	3, 76.1	20.45	.000
THIRDS	2, 49.7	3.50	.038
FORCE * SURFACE	6, 443	1.52	.17
FORCE * HEIGHT	4, 443	0.84	.50
FORCE * SUBJECT	25, 443	1.04	.42
SURFACE * HEIGHT	6, 443	0.65	.69
SURFACE * SUBJECT	78, 443	1.53	.004
HEIGHT * SUBJECT	52, 443	0.85	.75

Table 2: Estimated means for cube root volume by Force

FORCE	Mean	Std. error
HEAVY	.098	.008
LIGHT	.054	.008
CONTROL	.024	.008

Table 3: Estimated means for cube root volume by Surface

SURFACE	Mean	Std. error
MESIAL	.112	.009
DISTAL	.096	.009
LINGUAL	.014	.009
BUCCAL	.014	.009

Table 4: Estimated means for cube root volume by Thirds

THIRDS	Mean	Std. error
CERVICAL	.048	.008
MIDDLE	.053	.008
APICAL	.074	.008

5. Future Directions

The decrease in root length after intrusion in this study sample is already being investigated. The present sample is being compared to a new control group where no force was applied on either left or right side.

Further research extending the experimental period might be required to understand the incidence of root resorption when force duration is longer than 28 days and to investigate if two-dimensional and three-dimensional measurements show significant differences.

Separation of the subjects into different ethnic backgrounds groups might be relevant however this would require a substantial sample to overcome individual variations.