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INVESTIGATION OF THE ORTHODONTIC EXTRUSION OF ROOT FILLED INCISORS IN BEAGLES

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A thesis submitted in partial fulfilment of the requirements for the degree of

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SUMMARY

Crown-root fractures of the maxillary central incisor teeth in children, have been reported as a consequence of a traumatic injury. These teeth often suffer irreversible pulpal damage, and require endodontic treatment followed by a coronal restoration. Although crown-root fractured teeth represent a small percentage of total traumatic injuries to the dental structures, they present a unique treatment challenge for the paediatric dentist. This challenge is due to the subgingival and subosseous extension of the fractured crown, and the concomitant damage to the periodontium.

Treatment modalities for crown-root fractured teeth have varied from root canal therapy followed by surgical recontouring of the soft tissues, with or without osseous surgery, to the development within the last twenty years of a clinically acceptable orthodontic extrusion technique. This latter technique was designed to move the fractured root supragingivally to allow adequate access for a coronal restoration.

The aims of this study were to determine the biological effects of orthodontic extrusion upon a root filled, decoronated incisor tooth, including the determination of the most appropriate extrusion force, the most appropriate retention period, and any alterations to the periodontium. This study was carried out using a beagle dog model.

The upper right first lateral incisor in seven beagle dogs was chosen to undergo treatment because of its ease of access, and the ability of the adjacent teeth to provide sufficient orthodontic anchorage for it to undergo extrusion. This tooth was decoronated with a high speed bur to simulate a traumatic dental injury. It was subsequently endodontically treated. Fixed orthodontic appliances were used to extrude this tooth in five of the animals. The remaining two animals acted as control specimens and did not undergo orthodontic extrusion after completion of their root canal therapy.
treatment. After a specified period of orthodontic extrusion, with varying extrusive forces for each experimental tooth, and a specified retention period, the animals were sacrificed. One of the five experimental animals had a periodontal surgery procedure performed in lieu of retention. Clinically, gingivitis was present in the experimental and the control teeth.

The appropriate regions of the maxillary jaw from each animal were removed for investigation. A light microscope (LM) histological, and scanning electron microscopic (SEM) examination of the root filled incisor teeth was subsequently performed.

Radiographic examination of the extruded teeth illustrated a widened lamina dura. This feature was present after the initial active extrusion phase (4 to 6 weeks). This was observed to have resolved prior to sacrifice. This feature was not observed in the control teeth.

Variation of the initial extrusive force (30 to 120 grams) did not appear have any effect upon the final degree of orthodontic extrusion. Four of the five experimental teeth experienced extrusion greater than one millimetre during the active extrusion phase. Relapse was evident in four of the five experimental teeth. The only tooth that did not experience relapse was the tooth which underwent a periodontal surgery procedure in lieu of retention. This tooth also had the most favourable clinical appearance at the time of sacrifice, with the least amount of gingivitis and the greatest amount of tooth structure visible. The final amount of extrusion at sacrifice, experienced by the experimental teeth ranged from 0.2 to 0.9mm. The gingival margin was also observed to have moved occlusally with three of the experimental teeth.

The interproximal vasculature of the periodontal ligament in the experimental teeth showed changes in total vascular area and blood vessel size, when compared with the control teeth. When compared with the adjacent second lateral incisor the vascular area
of the root filled extruded tooth was slightly greater, as was the blood vessel diameter. There was no increase in the number of blood vessels for the extruded teeth when compared with either the control teeth or to the adjacent non root filled incisor. The periodontal ligament fibres of the experimental teeth appeared to have normal alignment in both scanning electron microscope and light microscope sections. Cervical root resorption was present in three of the five experimental animals. Histological examination confirmed the presence of cellular cementum deposition in the resorptive sites. No cementoclasts or osteoclasts were observed histologically. The predominant cellular structure within the periodontal ligament was the fibroblast. Cervical resorption was observed in teeth which underwent extrusion with heavy orthodontic forces. Cervical resorption was not evident in the control animals.

Orthodontic root extrusion in beagle dogs resulted in alterations to the periodontal ligament vasculature, including changes to blood vessel diameter and area. The deposition of cellular cementum towards the apex of the extruded teeth was also observed. The presence of cervical root resorption was observed histologically, and with scanning electron microscope examination. The presence of cervical resorption requires further investigation, to determine its aetiology and time of occurrence, and the possible consequences it may have on the extrusion procedure, if any.
STATEMENT OF AUTHORSHIP

The experiments performed in this thesis were carried out at Westmead Hospital Dental Clinical School. General anaesthesia of the animals was carried out in the operating theatres of the animal vivarium of the hospital. Animal maintenance and care was carried out with the assistance of the vivarium attendants. Specimen processing for scanning electron microscopy and light microscopy was performed in the Education Clinic of the Dental School.

This report contains no material which has been accepted for the award of any other degree or diploma in any university.

To the best of my knowledge and belief this report contains no material previously published or written by another person except when due reference is made in the text of the report.

PETER DAVID WONG
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1 INTRODUCTION AND AIMS OF THE RESEARCH PROJECT

1.1 DENTAL TRAUMA

The treatment of traumatic dental injuries represents one of the most difficult challenges for the paediatric dentist.

Andreasen and Andreasen (1988) have stated that "Dental traumatology has become the orphan of the dental profession, being almost totally neglected by the academic profession with respect to both clinical and experimental research".

Epidemiological studies from Copenhagen have demonstrated that approximately 50% of school children have traumatised either their primary or permanent dentition before school leaving age (Andreasen and Andreasen, 1988). Andreasen and Andreasen (1990) have shown that in many industrialised nations, almost every second child has been affected by a form of traumatic dental injury (figure 1-1) which can often compromise the function and aesthetics of the dentition.

1.1.1 Aetiology of Dental Trauma

Andreasen (1981) reported that dental injuries are infrequent during the first year of life but increase substantially with a child's initial efforts to move. The incidence of dental injuries has been reported to reach a peak just before school age, and consisted mainly of injuries due to falls, collisions, and bumps (Ravn, 1974).
Figure 1-1  Graph of Incidence and Prevalence of Dental Trauma in Copenhagen School Children. Epidemiological studies performed in Copenhagen have demonstrated that approximately 30% of children have traumatised their primary dentition and approximately 25% of children their permanent dentition by school leaving age. From Andreassen and Andreassen (1990).
Dental injuries can be caused by many traumatic events. These include the following:

i. **School accidents**
Fall injuries from the school playground or sporting activities at school (Andreasen, 1981).

ii. **Sporting injuries**
These injuries are related to football, basketball, hockey, and wrestling. Horseback riding has been classified as the third most prevalent source of sports injuries, recorded among ten registered sports activities (Andreasen, 1981).

iii. **Motor vehicle accidents**
Facial and dental injuries are commonly seen in the teenage group following motor vehicle accidents. Enforcement of seat belt laws in Australia has decreased the incidence of facial trauma in children (Hall, 1986).

iv. **Medical conditions**
Common medical conditions including epilepsy, and physically debilitating conditions such as Cerebral Palsy result in patients being more susceptible to dental injuries. This is because such patients are likely to suffer uncontrolled seizures or fall from the lack of motor control (Andreasen, 1981).

v. **Predisposition to Dental Injuries**
A predisposition to dental injuries can occur with increased protrusion of the maxillary teeth. Dental injuries can occur in a ratio of almost 2:1 for these children compared with children with a normal overjet or overbite (Andreasen, 1981).

vi. **Child abuse**
Sadly this group of children is prevalent in our society at a very much hidden level. Trauma received in these situations can involve dental injuries (Andreasen, 1981).
1.1.2  Prevalence of Dental Injuries

Battenhouse et al. (1988) have found the following incidence of dental injuries in a survey from the hospital outpatient clinic in a large children's hospital:

- Facial lacerations and abrasions: 58%
- Displacement, subluxation and tooth fracture: 35%
- Facial bones and temporomandibular joint injuries: 3%

Shultz (1988) confirmed that minor soft tissue injuries, are the most common form of dental trauma in children.

Infants have been reported to sustain most of their dental injuries from falls in the home (Galea, 1984). Boys were found to sustain injuries twice as often as girls. Results of traumatic dental injuries to children have varied from a 1:1 ratio of male to female, up to a 3:2 ratio of male to females sustaining dental injuries (Andreasen, 1981; Garcia-Godoy et al., 1979; Berkowitz et al., 1980).

1.1.3  Location of Dental Injuries

Most dental injuries, as reported by Hallet (1953) and Andreasen (1981), involve the maxillary central incisor teeth while the mandibular central incisor and maxillary lateral incisor teeth are less frequently affected.

Andreasen (1981) has observed that traumatic dental injuries usually affect a single tooth. However, it has been reported that certain traumatic events such as automobile accidents and sporting injuries may favour multiple dental injuries, including dentoalveolar injuries and facial fractures.

1.1.4  The Fractured Tooth

Crown fractures in children may involve the enamel, dentine or pulp. They are common dental injuries which can be diagnosed easily and treated accordingly (Andreasen and Ravn, 1972).
Root fractures of teeth are uncommon dental injuries (Andreasen and Ravn, 1972). The incidence of root fractures, has varied from 1% to 7% of total traumatic dental injuries (Ravn, 1974). Root fractures of teeth have been found to most likely occur after the crown of a tooth has fully erupted and the root fully matured. The surrounding structures at this period of dental development have firmly fixed the tooth into its socket, consequently dental trauma can lead to a root, crown, or crown-root fracture of a tooth.

Root fractured teeth are classified according to their site of damage, and it has been observed that the closer to the gingival margin the root fracture is, the poorer the prognosis for that tooth (Berkowitz et al., 1980; Tsamtsouris and Ko, 1985).

Root fracture healing has been reported to occur via the following three modes (Andreasen, 1981):

i. Union of fragments with calcified hard tissue.

ii. Interposition of connective tissue.

iii. Non union with associated inflammation and interposition of granulation tissue.

A characteristic feature of root fractured teeth is that the apical fragment can contain vital pulp tissue (Andreasen and Hjorting-Hansen, 1967; Jacobsen and Kerekes, 1980).

Crown-root fractured teeth as described by Heithersay (1973) are those teeth with fractures extending below the alveolar crest (figure 1-2). Heithersay and Moule (1982) reported that these teeth invariably require root canal therapy due to pulpal damage from the initial dental trauma.

The complex nature of the crown-root fractured tooth presents many problems and as such treatment difficulties for crown-root fractured teeth have included the following (Simon et al., 1978):
i. The inability to maintain a healthy periodontium due to bacterial leakage through the fracture site.

ii. The inability to adequately restore the fractured crown successfully.

Figure 1-2  Crown-root fracture of a maxillary left central incisor (clinical patient photograph).
Heithersay (1973) developed an orthodontic root extrusion technique as an alternative method for treatment of crown-root fractured teeth. Since that time the majority of reports have provided only empirical information based on the clinical performance of the technique.

Orthodontic extrusion of endodontically treated teeth has been indicated by Simon (1984) for the treatment of subgingival defects caused by root fractured teeth, root resorption, and root perforations. In these situations the subgingival defects are required to be brought supragingivally to allow access for definitive treatment.

Simon et al. (1980), were the first to report the histological changes associated with orthodontic extrusion of endodontically treated decoronated teeth. They used a dog model to perform their study. In their investigation, the extrusive force and the amount of relapse was not measured, however they did conclude that the extrusion technique was successful.

At present information is still required concerning:

i. The biological effects upon the periodontium following orthodontic extrusion of a root filled tooth.

ii. The most appropriate rate and force of extrusion for a crown-root fractured tooth.

iii. The most suitable retention period for these teeth.

1.2 AIMS OF THE RESEARCH PROJECT

1. To review the current literature concerning the orthodontic extrusion of root filled teeth.

2. For the author to develop skills in scanning electron and light microscope examination techniques.

3. To determine the clinical success of the orthodontic extrusion technique in a beagle dog using conventional bonded orthodontic appliances.

4. To investigate the cellular response of the periodontal ligament following orthodontic extrusion of endodontically treated teeth in beagle dogs.

Particular reference will made to:

i. Periodontal ligament adaptations.

ii. Dentine and cementum alterations.

iii. Vascular changes.

5. To determine the effect of differing extrusive forces on the rate of extrusion and the elevation of the gingival housing.

6. To provide more information on the appropriate retention period to prevent reintrusion of the extruded tooth.
2 CROWN-ROOT FRACTURED TEETH AND THEIR TREATMENT MODALITIES

The following chapter will review the aetiology and treatment regimes for crown-root fractured teeth, and the various treatment dilemmas that these unusual dental fractures present to the clinician.

2.1 CROWN-ROOT FRACTURED TEETH

Traumatic injuries to the teeth of children include crown and root fractures. Crown fractures, as reported by Tsamstouris and Ko (1985), can be easily diagnosed and treated accordingly. These authors have reported that root fractures are more likely to occur after the crown of a tooth has fully erupted. Crown-root fractures of teeth have been defined by Heithersay (1973), as "transverse root fractures in which the fracture line lies 1-4mm below the alveolar crest". This definition was restated by Heithersay and Moule (1982). Clyde (1965) illustrated the appearance of a crown-root fractured incisor (figure 2-1).

2.1.1 Aetiology of Crown-Root Fractured Teeth.

Heithersay and Moule (1982) have described that for anterior teeth, the force from a traumatic blow usually originates from a labial direction, at approximately right angles to the long axis of the tooth. These authors indicated that most fractures involving crown and root have greater loss of the tooth palatally than labially (figure 2-2).
Figure 2-1  Transverse oblique fracture of a maxillary incisor. A, fractured surface of the labial component; B, deepest extension of the palatal component. From Clyde (1965).

Figure 2-2  Crown fragment of a crown-root fractured incisor tooth (clinical patient photograph).
2.1.2 Prevalence of Crown-Root Fractured Teeth

Crown-root fractured teeth comprise a small percentage of total traumatic dental injuries. Andreasen (1970) found from a study in Copenhagen that crown-root fractured teeth occurred in 5% of the total number of patients examined for traumatic dental injuries. Davis and Knott (1982) found that crown-root fractured teeth occurred in 9.7% of traumatic dental injuries. Martin et al. (1990) found that crown-root fractured teeth occurred at 4.3% and 4.4% in two major teaching hospitals in Newcastle and Western Sydney respectively.

2.1.3 Healing Modalities of Root & Crown-Root Fractured Teeth

After root fracture of a tooth, the following modes of healing have been reported by Heithersay (1973):

i. Healing by hard tissue union of the fragments.

ii. Healing by connective tissue union of the fragments.

ii. Non union due to the interposition of granulation tissue between fragments resulting from pulpal necrosis of the coronal fragment.

2.1.4 Treatment Difficulties Associated with Crown-Root Fractured Teeth

There are certain problems involving the treatment of crown-root fractured teeth which extend below the alveolar crest:

i. Lack of Immobilisation

The proximity of the fracture line to the gingival crevice has provided extreme difficulties for the successful immobilisation of the coronal segment in crown-root fractured teeth, consequently hampering the ability of the tooth to achieve calcific union (Heithersay, 1973).

ii. Bacterial Contamination

This was reported to arise due to the lack of immobilisation, and proximity of the crown-root fracture to the gingival crevice.
Contamination may be immediate, or it may follow the regression changes associated with difficulties in immobilisation of the coronal segment (Heithersay, 1973).

iii. Periodontal Considerations
There has been difficulty in establishing a stable periodontal condition following the surgical intervention required to locate the subgingival crown margin, or to correct the differing gingival margin levels for crown-root fractured teeth. It has also been considered that surgical removal of adjacent alveolar bone can jeopardise the remaining alveolar bone support (Heithersay, 1973).

2.2 TREATMENT FOR CROWN-ROOT FRACTURED TEETH
In crown-root fractured teeth, the position and the circumferential extent of the fracture are both of considerable importance when deciding upon the most suitable treatment for these traumatic injuries. The severity of the fracture in a subgingival direction has been documented by Heithersay and Moule (1982) to be the most important factor influencing the treatment plan.

2.2.1 Restorative Procedures
Ellis (1960) advocated using the severed crown as the permanent restoration with a prefabricated metal post for retention (figure 2-3). A number of cases have been described using this technique (Lee, 1960). A disadvantage with this method as noted by Langdon (1968), was that the crown was rarely separated from the root by a single clean fracture line, but more often by a series of small fragments on the palatal aspect of the tooth. There is little evidence in the current literature to suggest that this method is being widely utilised as a clinical treatment procedure for crown-root fractured teeth.

An interesting technique was described by Clyde (1965). He used the fractured crown to facilitate an impression of the margins of the subgingival region. His technique
entailed taking an impression of the root canal with a brass post covered in impression compound, and then fitting the fractured crown over this. In this manner it was hoped that an accurate impression of the subgingival root fracture would be obtained. Inherent in this technique were the inaccuracies of any impression taken in impression compound and the difficulties of ensuring that the detached crown was adequately repositioned (figure 2-4).

Figure 2-3  Ellis' method for restoring crown-root fractured teeth. A, root filling; B, post; C, cement. From Ellis (1960).
Figure 2.4  Clyde's method of restoring crown-root fractured teeth. Impression stage. A, root filling; B, brass post; C, impression compound. From Clyde (1965).

2.2.2  Periodontal Surgery Procedures

One of the traditional methods of treating teeth with advanced subgingival dental caries or a traumatic injury such as a crown-root fracture that extended apically into the alveolar crest, was to expose adequate sound tooth structure with periodontal surgery. The subsequent soft and hard tissue surgery would often result in reduced alveolar bone support, an altered crown-root ratio, and poor aesthetic results (Potashnick and Rosenberg, 1982).

Prophet et al. (1964) recommended a localised gingivectomy, and associated surgical reduction of the alveolar crest, to permit access for restorative procedures, but only if the oblique fracture extended no more than two to three millimetres below the gingival margin.
Heithersay and Moule (1982) advocated that if the fracture line in a crown-root fractured tooth occurred deep on the palatal surface and did not involve the interproximal area, periodontal surgery was recommended as quite an acceptable form of treatment to uncover the osseous defect. Surgery in those situations involved a full thickness palatal flap and removal of bone from the palatal area to expose the fracture line, allowing thorough visual examination of the root surface to check for additional fractured spicules of tooth structure. A good bony contour should be created laterally and sufficient bone removed to ensure that the margins were still exposed after the flap had been replaced. As a localised palatal defect was difficult to clean, it has been recommended that these flaps be undermined laterally, past at least two adjacent teeth, to reduce the soft tissue thickness, and impart a good contour to the palatal tissues for oral hygiene purposes (Heithersay and Moule, 1982).

Where a crown-root fracture of a tooth occurred in the labial or interproximal areas, the amount and extent of possible tissue reduction was dependant upon the position of the lip line. Where the lip line was high, elongation of the crown following periodontal surgery would not be aesthetically pleasing. In clinical situations where the fracture was so far subgingivally that an adequate gingival contour could not be attained, or the fracture extended deeply into the interproximal or labial regions, surgery should not be attempted and alternative forms of treatment sought (Heithersay and Moule, 1982).

2.2.3 Intra-alveolar Transplantation
A surgical method for the extrusion of crown-root fractured teeth through intra-alveolar transplantation has been described by Tegsjo et al. (1978). The intra-alveolar transplantation method allowed direct inspection of the root, enabling the presence of previously undiagnosed cracks to be detected. Tegsjo et al. (1987) has stated that "a practitioner who has visually examined the root is in a better position to decided the most suitable course of treatment".
The technique described by Tegsjo et al. (1978) was as follows (figure 2-5):

i. Labial mucoperiosteal flap.

ii. Removal of labial bone from the apex of the involved tooth to locate it, and assess its overall condition.

iii. The removed bone was stored in saline for later use.

iv. After vertical luxation of the root through this buccal window, the original apical bone fragments were then placed back at the apex of the tooth and used as an apical stop.

iv. Root stabilisation with sutures and a pulpectomy was commenced.

Tegsjo et al. (1987) described the results of fifty seven teeth treated in this manner over a four year period. All of the teeth exhibited healing without ankylosis. They noted that two to nine weeks postoperatively, endodontic and or prosthetic treatment could be carried out. After this the teeth could continue to perform well in terms of aesthetics and function.

Intra-alveolar transplantation has been described as an alternative method of treating crown-root fractured teeth. Splinting was not thought to be necessary as the aim was to allow normal functioning to occur as soon as possible (Tegsjo et al., 1987).

The advantages of intra-alveolar transplantation can be summarised into the following four points (Tegsjo et al., 1987).

i. The opportunity to inspect and diagnose injuries to the periodontal membrane and cementum, and to detect cracks and fractures in the dentine.

ii. The possibility of limiting extrusion by rotating the tooth by one hundred and eighty degrees. In situations where the major part of the fracture was on the labial the tooth was rotated so as to place the fractured margin on the palatal to improve labial aesthetics.

iii. Opportunity to carry out endodontic treatment under aseptic conditions.

iv. Possibility of immediate treatment of a tooth with single or multiple crown-root fractures regardless of the adjacent teeth.
2.3 THE ORTHODONTIC EXTRUSION TECHNIQUE
As previously mentioned, this technique was initially described by Heithersay in 1973. Since then, numerous clinical reports and case studies have been documented, usually with pleasing results (Wolfson and Seiden, 1975; Simon et al., 1978; Ivey et al., 1980; Stern and Becker, 1980; Cooke and Scheer, 1980; Fournier, 1981; Lemon, 1982; Potashnick and Rosenberg, 1982; Hovland et al., 1983; Simon, 1984; Garrett, 1985; Johnson and Sivers, 1986). A histological study on extrusion of teeth in dogs has also been documented with acceptable results (Simon et al., 1980).

The goal of the orthodontic extrusion technique as defined by Heithersay and Moule (1982), was and is, to "raise the defect on the fractured root surface from within the alveolar bone to a position above the alveolar crest". Simon (1984) has stated that "orthodontic extrusion of a root filled fractured incisor is to allow the restoration of any subcrestal defect by elevating it to a point where access is no longer a problem".

Orthodontic extrusion has been defined as a clinical crown lengthening procedure and as such, the objectives of the technique can be listed as follows (Ingber, 1976a):

i. Exposure of sound tooth structure for the placement of adequate restoration margins.

ii. Increase of clinical crown dimensions thereby improving retention possibilities of a dental restoration.

iii. Providing improved maintenance of the biologic width by maintaining a healthy periodontium.

2.3.1 Orthodontic Extrusion Principles
Orthodontic extrusion elevates a root in its socket and also stretches the periodontal fibres of the tooth. As the movement is vertical, the root was not expected to move through bone or crush the periodontal ligament, hence no root resorption was anticipated (Simon et al., 1984). Simon et al. (1984) also noted that the gingival
attachment, or housing of the extruded tooth may or may not move with it. This was documented to be a function of how rapidly the root was extruded and how much force was used (Simon et al., 1984).

Tooth extrusion has been considered to be the easiest orthodontic movement to achieve, as it closely resembles natural tooth eruptive movements (Stern and Becker, 1980). Biggerstaff et al. (1986) have noted that a force of 20 to 30 grams was sufficient to cause the forced eruption of a single tooth. These authors have also noted that intrusion can be difficult to achieve. Thus in a situation where there are a number of orthodontic brackets in position, extrusion, will always occur prior to intrusion (Stern and Becker, 1980).

2.3.2 Indications for Orthodontic Root Extrusion

Orthodontic root extrusion has been indicated as the treatment of choice for the management of any cervical third root problem that involves or extends below the alveolar crest of bone by up to four millimetres (Simon et al., 1978; figure 2-6).

These problems can include:

i. Horizontal, cuspal, or shear root fractures.

ii. Cervical carious destruction of teeth.

ii. Cervical resorption, which may be external or internal resorption.

iv. Iatrogenic root perforations which occur subgingivally.

The technique should also be considered when poor aesthetics or a gingival defect would result from periodontal surgery and osseous recontouring (Simon, 1984).

Lythgoe et al. (1980) have reported that any abutment teeth to be used as anchorage for the orthodontic extrusion procedure should be healthy with no carious lesions or large restorations. The tooth to be extruded should also have had a successful root canal filling completed.
Figure 2-6 Indications for the use of root extrusion techniques. From Simon et al. (1978).
2.3.3 Contraindications for Orthodontic Root Extrusion

There are few contraindications to the orthodontic extrusion technique, however Simon (1984) listed three specific contraindications:

i. Insufficient root length after extrusion.

ii. Insufficient occlusal clearance to extrude the fractured tooth the desired amount.

iii. Possible periodontal complications due to damage to the gingival epithelium.

2.3.4 Advantages of the Orthodontic Extrusion Technique

The main advantage of the orthodontic extrusion technique was its conservative approach. By keeping the root within the alveolus, bone height could be maintained, consequently periodontal support was not compromised (Melhamm and Prescott, 1975; Simon, 1984).

Heithersay (1973) considered that it was difficult to establish a stable periodontal state following surgical intervention because of differing bone and soft tissue levels. Past methods of combined periodontal surgery and endodontics, for the treatment of crown-root fractured teeth have been considered unsatisfactory because:

i. The aesthetic result was usually poor due to the excessive clinical crown length on completion of treatment.

ii. Removal of the alveolar bone usually jeopardised the periodontal support of the adjacent teeth such that more harm could be instigated by the loss of important alveolar support.

With the root being maintained, there was no need to involve the adjacent teeth in the restorative procedure (Heithersay, 1973). Restoration of a single tooth has been considered to be more desirable than a fixed or removable partial denture for the following reasons (McDonald et al., 1982):
i. Adjacent healthy teeth do not require preparation as abutment teeth when restoration of a single tooth is carried out.

ii. Partial dentures encourage plaque retention which in turn promotes gingival inflammation.

iii. Single tooth retainers can be most aesthetic.

2.3.5 Disadvantages of the Orthodontic Extrusion Technique

The disadvantages of the orthodontic root extrusion procedure as listed by Simon (1984) are as follows:

i. Aesthetics of the temporary restoration may not have been acceptable to the patient.

ii. The procedure can be very time consuming.

iii. Periodontal surgery in certain situations is still necessary at the completion of the extrusion procedure.

2.3.6 Clinical Technique of Orthodontic Extrusion

Numerous clinical techniques have been developed to extrude a crown-root fractured tooth, though the principles described for each are essentially similar. Ingber (1976b) described the extrusion procedure as the "forced eruption" technique. A diversity of techniques have been necessary because the clinical situation varies in each individual situation. The appropriate technique selected, as described by Delvianis and Delvianis (1984), depended upon:

i. The availability of the adjacent teeth to provide support for the orthodontic appliances.

ii. The remaining tooth structure of the tooth to be extruded.

iii. The aesthetic demands of the patient.

In practice, a vertical extrusive force was placed onto the fractured tooth at the completion of its endodontic treatment. This vertical force was applied to the tooth to
move it in a coronal direction. Full or partial orthodontic banding may be chosen in relation to the clinical situation. Since Heithersay's original description in 1973, many reports of single extrusion cases have appeared in the literature, (Wolfson and Siden, 1975; Delvianis et al., 1978; Simon et al., 1978; Tofsky and Tsamstouris, 1979; Simon, 1984; Wong and Fricker, 1991).

Heithersay (1973) documented two similar techniques, for extruding a crown-root fractured tooth. One technique has been designed for a tooth with its crown in position, and one for a tooth without a crown (figure 2-7). Heithersay's technique involved the following procedures:

i. After completion of endodontic treatment, orthodontic appliances were applied to the anterior and posterior teeth, a temporary post was then cemented into the fractured tooth if necessary.

ii. Orthodontic forces were applied via the use of an activated multistrand orthodontic wire. The desired position of the root could usually be attained within four weeks.

iii. Heithersay also advised that the tooth be maintained in this position for a further six weeks to allow stabilisation of the periodontal fibres and the supporting bone.

iv. It has also been noted that minor periodontal surgery may be necessary to correct the final gingival contour.

v. Heithersay also considered it necessary that an orthodontic retainer be worn for a further six months to prevent subsequent reintrusion of the tooth.
Figure 2-7  Heithersay's original orthodontic extrusion technique (Labial view of maxillary incisors). Tooth 1 has a clinical crown. Tooth 2 has no crown. A, root canal therapy completed; B, post inserted; C, extrusion commenced; D, temporary post removed; E, final restoration completed. From Heithersay (1973).
A technique was described by Simon et al. (1978) and Simon (1984) utilising a rigid wire splint cemented to the adjacent teeth with composite resin instead of fixed orthodontic appliances as anchorage for orthodontic movement of crown root fractured teeth (figure 2-8). A small post was placed into the fractured tooth and activated with a small elastic thread attached to the rigid splint. These authors advocated a weekly recall to monitor the distance of extrusion of the crown-root fractured tooth. They considered that extrusion could be complete within one to three weeks, which contrasted to four weeks advocated by Heithersay (1973). Simon (1984) considered that a minimum of eight to twelve weeks of retention was necessary to prevent reintrusion of the orthodontically extruded tooth.

Melhamm and Prescott (1975) described a technique utilising the cementation of a post through the coronal segment of the fractured crown. The technique described by these authors was essentially the same as that of Heithersay (1973). These authors reported that after orthodontic movement was complete it was desirable to leave the extruded tooth in passive retention for a further six to eight weeks. Lythgoe et al. (1980) considered that extrusion could be achieved within one to two months and advocated a further retention period of eight weeks after active extrusion of the fractured tooth.

Other methods to extrude teeth with no clinical crown present have been developed. Lemon (1982) suggested cementing a temporary crown directly to the endodontic post and then attaching a bracket to the gingival third of the crown. Lemon (1982) also reported that in two weeks of active movement three to four millimetres of tooth extrusion could be expected. He also reported that retention was important to the reorganisation of the fibres of the periodontal ligament and recommended that one month of stabilisation per millimetre of extrusion be utilised.
Figure 2-8 Simon's orthodontic extrusion technique utilising composite resin and a rigid wire (Labial view of incisor teeth). A, fracture below the alveolar crest of bone; B, Endodontics completed, post placed, and horizontal wire bonded to the adjacent teeth. From Simon (1984).

The length of orthodontic therapy has been reported to be dependant upon the desired amount of extrusion required. Ingber (1976b) advocated that approximately one and a half weeks should be allowed for each millimetre of extrusion.

A technique was developed by Cronin and Wardle (1981) where the initial post and core used to extrude the tooth was also used as a permanent post and core upon completion of extrusion. The authors considered this technique advantageous as it avoided repeated enamel preparation of the fractured tooth and allowed a greater degree of aesthetic management. A ball retainer was incorporated on the labial surface of the post to allow placement of an orthodontic elastic, and over this was placed a temporary crown.
Cooke and Scheer (1980) illustrated certain aspects of the extrusion technique which they considered significant. These authors considered that reciprocal intrusion by anchor teeth, combined with the need to etch or band sound teeth were sufficient reasons to contemplate the use of removable orthodontic appliances to initiate extrusion of the tooth root. The ideal extrusive force was considered by Cooke and Scheer (1980) to vary between 0.7 and 1.5N\(^1\). Application of elastics by the patient was considered advantageous as it allowed the patient daily monitoring of the clinical situation. Fournier (1981) also considered the removable appliance advantageous as it reduced the secondary effects of anchorage such as intrusion on the adjacent teeth, and also reduced chairside time, reduced cost, and improved oral hygiene. Mandel et al. (1982) also considered the use of removable appliances as advantageous in the clinical environment as it was relatively inexpensive and easily managed by the patient. Ries et al. (1988) have documented the successful extrusion treatment of a tooth with subgingival carious involvement with the use of a removable appliance. A removable appliance was used in their case because the tooth being extruded was adjacent to an edentulous arch which was unable to provide anchorage.

A technique describing the utilisation of a segmental archwire and shoe loops was documented by Biggerstaff et al. (1986). In this technique, teeth on either side of the target tooth were used as stabilizers. The use of two shoe loops reduced the tendency for reciprocal forces to cause displacement of the stabilising teeth. Flexible, multistranded arch wires were advocated by Biggerstaff et al. (1986) to extrude crown-root fractured teeth. Multistranded archwires have a limited range of function thus the incorporation of T-loops and shoe loops extended the range of activity. Reciprocal forces must be transferred to the adjacent stabilising teeth without causing undesirable tooth movements while extruding the crown-root fractured tooth. Biggerstaff et al.

\(^{1}1.0N\) equals 100 grams.
(1986) considered that extrusion could be complete within four weeks. Two adjustments were required during the extrusion period.

It has been reported that the gingival margin may or may not move with the tooth. This according to Simon (1984) was dependant upon the rate and degree of extrusive force used. A study on two adult rhesus monkeys, by Batenhorst et al. (1974), found that during facial tipping and extrusion of incisor teeth, the attached gingival margin of all experimental teeth increased. In three clinical cases of root extrusion documented by Biggerstaff et al. (1986), it was observed that the alveolar crest moved with the epithelial attachment in each situation.

Wong and Fricker (1991) documented a case in a twelve year old girl who suffered a crown-root fracture of a maxillary central incisor. After the active extrusion period the gingival margin was found to move with the tooth. A labial gingivectomy and a palatally replaced flap were performed to correct the gingival architecture of the extruded tooth. A three month retention period was utilised for this patient. Twelve months post treatment the tooth was reported to be in its original position and functioning well.

In summary the treatment of crown-root fractured teeth is diverse and situation dependent. The crown-root fractured tooth presents a complex treatment dilemma concerning the most suitable coronal restoration, and also the most favourable method to treat the gingival alterations. The orthodontic extrusion technique has been developed by Heithersay (1973) as an alternative treatment method for crown-root fractured teeth. A survey of the literature illustrates differing views on the rate and force of extrusion (table 2-1). The orthodontic extrusion technique is conservative and easy to manage. As Simon (1984) has reported, the extrusion technique can be utilised for extrusion of teeth with cervical resorption and subgingival caries, as well as for crown-root fractured teeth.
<table>
<thead>
<tr>
<th>Author</th>
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<th>Extrusion period</th>
<th>Extrusion force</th>
<th>Periodontal surgery advocated</th>
<th>Retention period</th>
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<td>Orthodontic bands</td>
<td>1-2 months</td>
<td>not mentioned</td>
<td>not mentioned</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Simon et al. (1978), and Simon (1984)</td>
<td>composite resin wire splint and elastic thread</td>
<td>1-3 weeks</td>
<td>not mentioned</td>
<td>Periodontal surgery possible</td>
<td>8-12 weeks</td>
</tr>
<tr>
<td>Biggerstaff et al. (1986)</td>
<td>Orthodontic bands</td>
<td>4 weeks</td>
<td>not mentioned</td>
<td>May be required</td>
<td>not mentioned</td>
</tr>
</tbody>
</table>

Table 2-1  Summary of the various treatment requirements for orthodontic extrusion of root filled teeth. Table compiled from information of past authors as listed.
3 THE TOOTH SUPPORTING STRUCTURES AND THEIR RELATIONSHIP TO THE CROWN-ROOT FRACTURED TOOTH

To understand the biological changes associated with the orthodontic extrusion procedure for a crown-root fractured tooth, a brief review of the periodontium will provide an overview of the histological and cellular alterations which may occur.

3.1 THE PERIODONTAL LIGAMENT, STRUCTURE & FUNCTION

The periodontal ligament has been described as the connective tissue interface separating a tooth from its supporting bone. Each tooth is attached to the adjacent alveolar bone by a heavy collagenous supporting structure, the periodontal ligament. Under normal circumstances, the periodontal ligament occupies a space approximately 0.5mm in width around all parts of the root (Proffit, 1986).

The periodontal ligament has been described as a viscoelastic structure. Light continuous forces (habits such as thumb/digit sucking, tongue posture, tooth eruption, or orthodontic therapy) have been described as highly efficient in displacing the periodontal ligament and moving teeth (Weinstein, 1980; Roberts and Chase, 1981).

The periodontal ligament consists of a fibrous stroma in a gel of ground substance containing cells, blood vessels and nerves. The fibrous stroma consists primarily of collagen with very small amounts of oxytalan fibres, and the cells have been reported to be mainly fibroblasts (Berkovitz, 1990).
3.1.1 Fibrillar Component of the Periodontal Ligament

Shackelford (1971) examined the dog periodontium under the scanning electron microscope. Mandibles were removed from four mongrel dogs and placed in formalin. At low magnification with the scanning electron microscope, Shackelford found that as distinct structures, fibre bundles passing from alveolar bone to cementum were not evident. Also no evidence of ligament cellularity could be visualised. The outstanding feature of Shackelford's study was a fibre meshwork identified under high power as the "indifferent fibre plexus". This plexus had individual components running in all directions to form a close meshwork. The presence of an indifferent fibre plexus, forming anastomosing relationships with the principal fibres, was thought to explain the adaptability of the periodontium during the process of tooth movement (Sicher, 1942).

Shackelford (1971) found that the indifferent fibres and the plexus which they form could be seen in cross sections and longitudinal sections of the periodontal ligament. He found the indifferent fibres enter and leave the principal fibre bundles so as to form anastomoses between adjacent fibre bundles. The plexus was described as extending from bone to cementum and was seen to be rarely interrupted except for the passage of blood vessels and nerves through the periodontium. The principle fibres were mostly obscured by the overlying indifferent fibres throughout the entire width of the periodontal ligament. He also found in decalcified sections that Sharpey's fibres were partially exposed and could be seen entering bone and cementum. In selected areas the indifferent fibres appeared to be pulled away from the principal fibre bundles. Each perforating indifferent fibre was found to consist of a closely packed fibre bundle.

Recently it has been suggested that the indifferent fibre plexus is probably a preparation artefact (Sloan et al., 1976). Ten Cate (1976) has also questioned the existence of the indifferent plexus. He considered that this plexus was attractive to explain movements such as tooth eruption and tooth rotation though it was not adequate enough to explain
how the ligament remodelled during movement of the tooth in a mesial or distal direction. Edwards (1968) stated that the indifferent plexus did not exist.

A study of the human incisor periodontium by Svejda and Skach (1973) found that in transverse and longitudinal sections the periodontal ligament was filled by two fibre systems. They discovered a surprisingly large number of fine fibres running through the periodontal space in different directions and largely filling it in the form of a dense matted tissue. On the surface of the bone and cementum there was a ridge composed of a dense fibre network, from which the individual fibres led into the cementum or bone. Blood vessels surrounded by compact bone ran through the interdental septum.

Ten Cate (1985) observed that the vast majority of the collagen fibrils in the periodontal ligament of humans are arranged in definite and distinctive fibre bundles; these fibre bundles have different orientations in different parts of the periodontal ligament (crestal, horizontal, oblique, apical, interradicular). Ten Cate (1985) has described that each bundle resembled a spliced rope; individual fibres were continually remodelled while the overall fibre maintained its architecture and function (figure 3-1).

The precise location of the shear zone within the periodontal ligament which allowed a tooth to erupt with respect to the alveolar bone has not been fully established (Sloan, 1982). The absence of any localised zone within the middle of the periodontal ligament showing a higher rate of turnover compared with other zones has been demonstrated by autoradiographical studies. It has been found that there is a rapid and uniform uptake (and subsequent uniform loss) of label across the entire width of the periodontal ligament (Rippin, 1976; Beertsen and Everts, 1977).
Figure 3-1  Arrangement of the principal fibre groups of the periodontium. A Coronal view shows the classic fibre groups. B Longitudinal view shows the fibres of the gingival ligament. From Ten Cate (1985).
3.1.2 Collagen

Collagen is generally regarded as being arranged in the form of principal bundles, having different orientations in different parts of the ligament. These fibre bundles form branching networks, and have a complex three dimensional network with overlapping of the bundles in adjacent layers (Sloan, 1979; Sloan, 1982). Collagen fibre bundles form branching networks which course around the neurovascular bundle. Close to cementum the bundles are 3 to 10 μm in diameter, while near the alveolar wall they are less numerous but thicker, with a diameter of 10 to 20 μm (Zwarych and Quigley, 1965; Shackleford, 1971; Sloan, 1982). Collagen in different regions along the length of a tooth may have different turnover rates. Perera and Tonge (1981) reported that the average half lives for collagen in the apical and crestal regions of the periodontal ligament were approximately 2.5 and 6.5 days respectively in young rats with erupting molars. In adult rats these values were approximately seven and eleven days respectively (Rippin, 1978).

Biochemically, the main type of collagen found in the periodontal ligament has been documented to be type I collagen. Twenty percent of the ligament collagen is type III collagen (Butler et al., 1975). The presence of type XII collagen has been demonstrated in the periodontal ligament, and as yet its functional significance is undetermined (Dublet et al., 1988).

Biochemical and autoradiographical techniques have shown that periodontal collagen has one of the most rapid turnover rates in the human body (Sodek and Ferrier, 1988). The significance of this high turnover rate is not yet understood, although it may be associated with the need for rapid adaptation of the tissue, e.g. during tooth movement (Kanoza et al., 1980). Collagen turnover rates appear to be increased when eruption is reactivated following the extraction of opposing teeth (Rippin, 1976; Kanoza et al., 1980). When collagen turnover is increased following reactivation of tooth eruption caused by extraction of opposing teeth, the effect is seen for type I and type III collagen.
(Kanoza et al., 1980). Duncan et al. (1984) using a mouse organ culture system containing three molars reported that the application of orthodontic loads resulted in a significant increase in the proportion of type III collagen.

3.1.3 Oxytalan Fibres

Oxytalan fibres in the periodontal ligament have been reported to lie parallel to the root surface (Fullmer et al., 1974). These form a three dimensional network, extending from the cementum to the peripheral blood vessels, (Sims, 1975; Sims, 1976). Bowling and Rygh (1988) noted that this meshwork exhibited a predominantly apico-occlusal orientation with a laterally connecting system of fine fibrils. They also found that there was a close relationship between the oxytalan fibres and the blood vessels in the periodontal membrane and they also found oxytalan fibres in the walls of blood vessels. At the light microscopic level, oxytalan fibres have been noted to range in diameter from 0.5 to 2.5 μm (Simpson, 1967). Follin et al. (1986) found that oxytalan fibres were arranged parallel to the root surface, except in the apical part where they were arranged almost perpendicular to the root surface.

It has been suggested that oxytalan fibres play a role in tooth support, increasing the rigidity of the periodontal ligament (Edmunds et al., 1979; Jonas and Riede, 1980). They are considered to serve as guides for cell migration during tooth eruption (Beertsen et al., 1974). They are thought to also act as part of a mechanoreceptor system which modulates the behaviour of vessels within the ligament, either directly or by the production of a more general neural response (Sims, 1973; Sims, 1977; Sims, 1983).

3.1.4 Cellular Component of the Periodontal Ligament

Fifty percent of the volume of the periodontal ligament (excluding blood vessels) has been reported to be occupied by cells (Beertson and Everts, 1977; Shore et al., 1984). Species differences have been reported to occur. The cellularity for sheep periodontal
ligament is considerably lower than 50%, when compared with humans (Berkovitz 1985). Also there has been evidence of decreased cellularity with age reported in humans (Grant and Bernick, 1972).

In the human, fibroblasts are described as ovoid or flattened (Fullmer, 1967) Fibroblasts have also been described as fusiform and elongated (Garant and Cho, 1979). Evidence from autoradiographical studies have indicated that periodontal fibroblasts move occlusally at a rate equal to that of tooth eruption (Beertson, 1975). Ten Cate et al. (1976) discussed the role of the fibroblast in the remodelling of the periodontal ligament during physiological tooth migration. They found that fibroblasts exhibited features one might expect to find in a cell actively involved in the synthesis and secretion of protein. It was discovered that the fibroblast was capable of degrading and synthesising collagen simultaneously, and was also noted to be able to control collagen remodelling (Ten Cate et al., 1976). Periodontal fibroblasts are also generated throughout life (Berkovitz, 1990).

Remodelling and recontouring of the bony socket and cementum of the root is constantly being carried out, as a response to normal function. As osteoblasts and cementoblasts of the periodontal ligament become incorporated into alveolar bone and cementum, replacement cells must be provided within the ligament to permit osteogenesis and cementogenesis to continue. It is not known whether these fibroblasts, cementoblasts, and osteoblasts all arise from a common precursor or whether each cell type has its own precursor cell (Berkovitz, 1990).

Dividing cells in the periodontal ligament have been located predominantly paravascularly, and migrate towards the bone and cemental surfaces, (McCulloch and Melcher, 1983).
3.1.5 The Ground Substance

The ground substance of the periodontal ligament has been estimated to be 70% water. Ten Cate (1985) reported that this composition was thought to have a significant effect on the tooth's ability to withstand stress loads. Berkovitz et al. (1981) reported that there was considerably more ground substance within collagen fibre bundles in areas subjected to compression (50%) compared with areas subjected to tension (27%).

3.1.6 Vasculature of the Periodontal Ligament

The main blood supply to the periodontal ligament is from the dental arteries. These arteries pursue an intraosseal course and give off alveolar branches that ascend within the bone as interalveolar arteries. Numerous branches arise from these vessels to run horizontally, penetrate the bone lining the alveolus, and enter the periodontal ligament space. As they enter the periodontal ligament they are called perforating arteries (Ten Cate, 1985). The vasculature of the periodontal ligament not only has a nutritive role but has been implicated in the support mechanisms of the tooth (Moxham and Berkovitz, 1982).

The arterial supply to the periodontal ligament is derived from three primary branches of the alveolar arteries (Lindhe, 1984):

i. the dental

ii. the interradicular

iii. the interdental.

Venous drainage channels accompany their arterial counterparts. The average size of these channels have been reported to be 28μm (Lindhe 1984).

The vessels of the ligament are thin walled and tend to run parallel to the long axis of the root. Values for density of blood vessels in the ligament vary according to tooth type and site; from about 7% in the adult mouse molar (Gould et al., 1977), up to about 50% near the base of the growing rat incisor (Moxham et al., 1985). There has also been
growing evidence that vascularity can vary between eruptive and post eruptive phases of tooth development (Moxham et al., 1987).

The blood supply to dogs was described by Perint (1949). The maxilla, and maxillary teeth were supplied by branches of the internal maxillary artery. The posterior superior artery supplied branches to the antral mucosa and the molar roots as well as the premolar teeth. The superior anterior alveolar artery supplied the nasal mucosa and anterior teeth. The superior labial artery supplied the gingiva and the labial frenum, and the palatine artery also contributed to the supply.

Soloviev (1970) found that the periodontal ligament of the dog was supplied by arteries that penetrated from the interalveolar septa. These arteries gave off numerous capillaries that intertwined around the larger vessels to form a vascular network (Soloviev, 1970; Ichikawa et al. 1976). Veins and arteries were found to run vertically (Ichikawa et al., 1976). Caranza et al. (1966) found that the blood vessels were closer to bone than to cementum. The layer closer to the tooth gave off many hairpin shaped capillary loops (Kindlova and Matena, 1962). Kishi and Takahashi (1977) supported this finding noting the presence of thick circular orientated vascular bundles were in the layer closer to bone. Egelberg (1966) reported that the dentogingival junction in the dog had a distinct layer of blood vessels close to the crevicular epithelium, also the diameters of the blood vessels ranged from 7μm to 40μm. No capillary loops as reported by Kindlova and Matena (1962) were present.

Weekes (1983) reviewed the various classification systems for blood vessels in the periodontal ligament. He made a synopsis of criteria to be used for classification of replicated blood vessels (table 3-1). Most of the studies that he used were from vascular casting studies. The classification he proposed provides us with an impression of the dimensions of the vessels within the periodontal ligament.
### Table 3-1

**Synopsis of criteria used for classification of replicated blood vessels.** From Weeks.

<table>
<thead>
<tr>
<th>VESSEL CATEGORY</th>
<th>ENDOTHELIAL CELL IMPRINTS</th>
<th>SHAPE OF LUMEN</th>
<th>PATTERN OF BRANCHING</th>
<th>INTERNAL DIAMETER RANGE (MICROMETRES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterioles</td>
<td>Cells have an oblong shape with spindle shaped or ovoidal nuclei; imprints oriented along direction of flow. The nuclear imprints show microvillus protrusions.</td>
<td>Straight but wavy below the site of branching; Round cross-section.</td>
<td>Relatively few branches which have the main trunk almost at right angles.</td>
<td>50 - 100</td>
</tr>
<tr>
<td>Terminal Arterioles</td>
<td>As for arterioles</td>
<td>As for arterioles</td>
<td>As for arterioles</td>
<td>8 - 50</td>
</tr>
<tr>
<td>Capillaries</td>
<td>No characteristic imprints have been described in the literature, although the endothelial cell number from two to five around the circumference.</td>
<td></td>
<td>Arise as side branches of an arteriolar-venular bridge or a terminal arteriole and end at a point where joined by an inflowing tributary to form a postcapillary venule. May not all be patent at any one time.</td>
<td>4 - 7</td>
</tr>
<tr>
<td>Postcapillary Venules</td>
<td>Cells have a random shape with circular or oval nuclear imprints which have no microvillus protrusions.</td>
<td>Slightly flattened cross-section</td>
<td>Receive blood from capillary networks. Diameter is twice that of capillaries and they are three times more numerous branches join at more acute angle than arterial branches. Could comprise the distal part of an arteriolar-venular bridge.</td>
<td>8 - 30</td>
</tr>
<tr>
<td>Collecting Venules</td>
<td>As for postcapillary venules</td>
<td></td>
<td>Receive blood from postcapillary venules and empty into small veins. Average diameter is three times that of arterioles but each has the same number of branches.</td>
<td>30 - 50</td>
</tr>
<tr>
<td>Small Collecting Venules</td>
<td>As for postcapillary venules</td>
<td></td>
<td></td>
<td>50 - 300</td>
</tr>
</tbody>
</table>
3.1.7 Cementum

Cementum has been described as a hard connective tissue, very much like bone, which covers the roots of the teeth. Its main function is the attachment of the fibres of the periodontal ligament to the root surface of teeth (Ten Cate, 1985).

The deposition of cementum continues throughout life. The vitality or the functional efficiency of cementum may be reduced greatly or even be lost as a result of alteration in functional forces. Cementum responds only by cementoblastic activity in which new functionally competent cementum is deposited over less vital cementum (Genco et al., 1990).

Various conditions, especially trauma, produce resorption of cementum (Genco et al., 1990). Such conditions producing resorption include the following

i. Extraordinary masticatory forces including parafunctional habits such as bruxism, and pen biting.

ii. Excessive orthodontic forces produced by removable or fixed appliance therapy.

iii. Disease states such as hypophosphatasia can lead to cementum resorption.

When these stimuli persist for prolonged periods, the area and depth of destruction may even reach into dentine. An area of cementum dissolution is characterised by surface concavities, with or without cementoclasts. With abatement of the resorbing stimuli cementogenic activity is resumed; leading to the production of cellular or acellular cementum, or a combination of both (Genco et al., 1990).

3.1.8 Alveolar Bone

The alveolar process is that part of the maxilla and mandible which contains the sockets for the teeth, and consists of outer cortical plates, a central spongiosa, and bone lining
the alveolus. The cortical plates and the bone lining the alveolus meet at the alveolar crest, usually 1.5 to 2.0mm below the level of the cementoenamel junction of the tooth it surrounds (Ten Cate, 1985).

Proffit (1986) has observed that with orthodontic extrusion of vital teeth, as long as the extrusive forces are reasonable and the rate of extrusion is not excessive, then alveolar bone will accompany movement of the tooth.

3.1.9 The Biologic Width

The healthy periodontium has been noted to exhibit constant dimensions of the combined supra-alveolar connective tissue and the junctional epithelium. These together are known as the biologic width, measuring slightly in excess of two millimetres (Ingber et al., 1977).

The junctional epithelium forms a collar around the tooth and is generally wider at the floor of the gingival sulcus and tapers as it progresses apically. The junctional epithelium has a high rate of cell turnover (Ten Cate, 1985).

In practice the biologic width represents the distance between the base of the gingival crevice and the alveolar crest (figure 3-2).

The biologic width has been reported to be of particular importance when considering the restoration of a tooth whose fractured or carious margin is at or below the level of the alveolar crest. If the tooth is restored regardless of the biologic width, a periodontal problem can remain in which the supra-alveolar connection of periodontal fibres is absent, leaving a gingival pocket of excessive depth. This can subsequently result in periodontal inflammation and bone resorption (Stern and Becker, 1980).
Figure 3-2  Diagrammatic representation of the cervical area of a tooth illustrating the biologic width. From Stern and Becker (1980).
The biologic width can be maintained in patients with crown-root fractures via two methods:

i. **Surgically lowering the alveolar crest by 2-3mm**
   
   This has not always been advisable because altering the bony architecture can be unfavourable to the adjacent teeth. An elongated clinical crown can be aesthetically unacceptable (Stern and Becker, 1980).

ii. **Orthodontically extruding the tooth**
   
   This technique seeks to maintain the biologic width as the entire tooth-supporting unit (periodontal fibres and bone) is moved coronally in relation to the adjacent teeth. This can lead to the generation of new bone (Stern and Becker 1980).

A healthy attachment apparatus has been considered essential for the retention of teeth and the protection of the subcrevicular tissues from communication with the oral environment. The biologic union of the junctional epithelium and the supracrestal connective tissue with teeth must be protected because it can easily be compromised by mechanical or bacterial trauma (Block, 1987).

### 3.2 RESTORATION MARGIN

When restoring crown-root fractured teeth following orthodontic extrusion, authors including Waerhaug (1978), and Newcombe (1974), have considered it essential to keep all restorations at or above the gingival margin and in a supragingival position when practical.

Wilson and Maynard (1981) cautioned against extending restorations too far subgingivally because the epithelial attachment could be damaged. Eissman *et al.* (1971) have recommended that restorations not be placed at or near the alveolar crest and that there must be at least two millimetres of root surface between the alveolar crest...
and the restoration, to provide for the biologic width. Block (1987) highlighted the importance of placing restorations no deeper than 0.5mm into the gingival sulcus so that they could be reached by the patient's oral hygiene methods.

3.3 PERIODONTAL SURGICAL PROCEDURES

After orthodontic extrusion of a crown-root fractured tooth it may still be necessary to correct the gingival defect with a periodontal surgical procedure. Stern and Becker (1980) declared that "it is imperative that the biologic attachment between tooth, bone, and gingiva not be encroached". McDonald et al. (1982) advocated an internal bevel incision for the periodontal surgical procedure. They reported that the external bevel incision was less predictable, and caused greater postoperative discomfort. Their reasons for an internal bevel incision include the following:

i. An internal bevel produces a thinner flap on the gingival margin.

ii. It allows the flap to be neatly reflected for adequate visualisation of the underlying bone.

iii. An internal bevel incision also provides retention of keratinised gingival tissue.

A technique was designed by Kozlovsky et al. (1988), to prevent the surgical phase of crown lengthening procedures after orthodontic extrusion of teeth. Repeated circumferential intrasulcular incisions were performed during the extrusive tooth movement phase. A clinical study performed on three patients by these authors found the technique to be completely successful. The authors felt that this technique was advantageous as post extrusion surgery could be avoided, however a small surgical procedure was necessary two to three times during the extrusion period.

Biggerstaff et al. (1986) observed that procedures to surgically lower the alveolar crest by two to three millimetres after orthodontic extrusion are essential and beneficial, and should be distinguished from routine crown lengthening procedures because the latter
often compromise the aesthetic appearance of the restoration and entail excessive removal of bone.

In summary the periodontal ligament is a complex structure consisting of cellular structures, collagen fibres, alveolar bone, blood vessels, and cementum. It undergoes numerous alterations during tooth movement and at present the exact mechanisms of such alterations are undetermined. The gingival crevice is of vital significance to the health of the tooth and the periodontium and when restoring teeth with subgingival margins, as in crown-root fractured teeth, the "biologic width" must be maintained.
Orthodontic movement of teeth has been based upon the principle that if prolonged pressure is applied to a tooth, movement of that tooth will occur as the bone around the tooth remodels (Proffit, 1986).

The response to sustained force against the teeth is a function of force magnitude. Heavy forces lead to rapidly developing pain, and necrosis of the cellular elements within the periodontal ligament, and the phenomenon of "undermining resorption", of alveolar bone near the affected tooth. Lighter forces are compatible with survival of the cells within the periodontal ligament and this leads to a remodelling of the tooth socket by a relatively painless "frontal resorption" phenomenon (Proffit 1986).

4.1 BIOLOGIC CONTROL OF TOOTH MOVEMENT

There are two possible control mechanisms reported that affect tooth movement; the "piezoelectric theory", and the "blood flow theory" (Proffit 1986).

4.1.1 The Piezoelectric Theory

The piezoelectric theory relates tooth movement in part, to changes in bone metabolism controlled by the electric signals produced from the bending and flexing of the alveolar bone (Baumrind, 1969). In tooth movement, undermining resorption, soft tissue inflammatory reactions, and tension on the periodontal ligament complex all produce a piezoelectric response. Proffit (1986) has stated that the piezoelectric effect may be significant for normal skeletal function, but probably has little to do with the response to orthodontic tooth movement.
4.1.2 The Blood Flow Theory

The blood flow theory relates tooth movement to cellular changes produced by alterations in blood flow through the periodontal ligament. In this theory an alteration in blood flow within the periodontal ligament is produced by the sustained pressure that causes the tooth to shift position within the periodontal ligament space, compressing the ligament in some areas while stretching it in others. Blood flow is decreased where the ligament is compressed, while it is maintained or increased where the periodontal ligament is under tension (Proffit, 1986).

If a tooth is exposed to a continuous load as in orthodontic tooth movement, disruption of the blood vessels can occur (Lilja et al., 1981). A study on the behaviour and role of blood vessels incident to experimental tooth movement was performed in rats (Rygh et al., 1986). The maxillary right first molar in male Wistar rats was moved mesially by means of a fixed appliance with an active spring for either 2, 7, 14, or 28 days. Particular attention was focused on areas of tension and of pressure. Extensive breakdown of collagen was observed in pressure areas undergoing frontal resorption and in areas of tension with vascular invasion. Intense vascular activity was found within the periodontal ligament and within the alveolar bone.

4.2 ORTHODONTIC TOOTH MOVEMENT

Orthodontic tooth movement is reported to be a complex phenomena that involves regulation of a number of biological activities (Norton and Burstone, 1989; figure 4-1).
Figure 4-1  Cascade of activities that must be regulated in root movement. From Norton and Burstone (1989).
Reitan (1967) reported that the determining factors in smooth and uniform tooth movement were:

i. The forces applied in orthodontic tooth movement.

ii. The patient's age, growth changes, and individual variations in their anatomic environment.

4.2.1 Comparison Between Animal and Human Tissue During Orthodontic Tooth Movement.

In attempting to correlate the relationship between human and dog reactions following orthodontic tooth movement an understanding of the difference between the species is valuable. It is generally stated that the alveolar bone of animals is denser than those of corresponding human structures (Reitan and Kvam, 1971). The alveolar bone of young humans usually contains large marrow spaces, open clefts and canals, whereas the labial and lingual bone plates in the dog are usually dense and thick Reitan (1951). Examination of the supra-alveolar fibres of the periodontal ligament revealed certain differences related to thickness and distribution of the marginal tissues of the upper first molars between man, monkey, dog, and rat (Reitan and Kvam, 1971; figure 4-2).

During orthodontic movement in the rat, hyalinised zones are produced readily and quite early. In humans as well as in the monkey and dog, tooth movement in a mesial or distal direction is generally followed by a more rapid undermining resorption than observed in a labial or lingual movement. When orthodontic forces are applied to the monkey and dog, undermining resorption starting in marrow spaces is frequently observed. It has also been illustrated in animal studies that provided a chain of osteoblasts and a thin layer of osteoid exist, tension will rapidly increase the thickness of these pre-existing structures and deposition of new bone will occur after a period as short as twenty five to thirty hours (Reitan and Kvam, 1971). Sharpey's fibres have been found in dogs as well as humans (Ruth, 1953). Reitan and Kvam (1971)
considered that differences do exist between different species yet results obtained in dogs and monkeys appear similar to those in humans.

Figure 4-2  Marginal and supra-alveolar tissues in various animals. The marginal and supra-alveolar fibres are conspicuous in the monkey and the dog, and less well developed in the rat. In man the supra-alveolar fibres are readily seen following tipping of the experimental tooth. Alveolar bone (AB), Supra-alveolar fibres (SA), Dentine (D). From Reitan and Kvan (1971).

Speelman and Collaert (1990) reviewed the role of the beagle dog in periodontal research. They found that for the beagle dog increased occurrence of root resorption, the better healing capacity of the dental tissues, and the progression rate of periodontitis were differences that existed when compared with the human. They felt that knowledge obtained in beagle dogs has implications for humans.
4.2.2 Comparison Between Animal and Human Tissues Following Endodontic Treatment

In relation to the use of animals for endodontic purposes it has not always been possible to produce experimentally the various pathological changes which affect human teeth (Barker, 1970). The animal of choice is therefore one in which the tissue response bears a similarity to the human (Barker, 1970). Endodontic experimentation which produced healing in the dog can be taken to suggest acceptable therapy in man (Orban, 1933). Barker (1970) performed a series of endodontic procedures in dogs and concluded that the results obtained from endodontic treatment in these animals could be extrapolated to the human subject.

4.3 THE EFFECT OF ORTHODONTIC FORCES ON THE PERIODONTAL LIGAMENT

Norton and Burstone (1989) reported an apparent pattern in the reaction of the periodontal ligament and alveolar bone to the amount and duration of tensitional stress. After a period of orthodontic forces applied to teeth they observed increasing vascularisation, cell proliferation, fibre formation, and osteoid apposition on the bone surface. Proffit (1986) has reported that when the blood supply is cut off to an area within the periodontal ligament a sterile necrosis occurs. Such an area has traditionally been referred to as "hyalinised". Rygh (1974) has found that most, if not all of the damaged material in the hyalinised zone is removed before repair is initiated. After several days osteoclasts differentiate within the adjacent bone marrow spaces and begin an attack on the underside of the bone immediately adjacent to the necrotic periodontal ligament area. This process was described as "undermining resorption" since the attack was from the underside of the lamina dura (Proffit, 1986; Norton and Burstone, 1986).

The optimal force levels for orthodontic tooth movement should be high enough to partially but not completely occlude blood vessels in the periodontal ligament. In this manner it was hoped that hyalinisation could be prevented (Proffit, 1986; Table 4-1).
Both the amount of force delivered to a tooth and also the area of the periodontal ligament over which that force is distributed are important. It is necessary to specify the type of tooth movement as well as the amount of force when determining optimal force levels for orthodontic purposes (Proffit, 1986).

Orthodontic extrusion movements ideally would not produce any areas of compression within the periodontal ligament, only tension. Proffit (1986) noted that this was more theoretical than practical since if the tooth was tipped slightly at all while being extruded, areas of compression would be created.

<table>
<thead>
<tr>
<th>Type of movement</th>
<th>Force (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tipping</td>
<td>50-75</td>
</tr>
<tr>
<td>Translational</td>
<td>100-150</td>
</tr>
<tr>
<td>Root uprighting</td>
<td>75-125</td>
</tr>
<tr>
<td>Rotation</td>
<td>50-100</td>
</tr>
<tr>
<td>Extrusion</td>
<td>50-100</td>
</tr>
<tr>
<td>Intrusion</td>
<td>15-25</td>
</tr>
</tbody>
</table>

Table 4-1 Optimal forces for orthodontic tooth movement. Smaller values appropriate for incisor teeth, higher values for posterior teeth. From Proffit (1986).

4.4 THE FIBRES OF THE PERIODONTAL LIGAMENT

The periodontal ligament has been reported to remodel during physiologic tooth movement (Ten Cate et al., 1976). Edwards (1968) proposed that the remodelling of the periodontal ligament occurred by the following methods:

i. Progressive osteogenic activity and cementogenesis play an active role in the shortening of extended fibres and in the reattachment of new fibres developed during tooth movement.
The stretching of the wavy collagen fibres and reorientation of their directional morphology could permit a certain amount of tooth movement.

To explain the orderly dissolution and synthesis of collagen Ten Cate et al. (1976) proposed the fibroblast to be capable of both synthesising and degrading collagen at any one time.

In examining orthodontic rotation it has been noted that the supracrestal connective tissue fibres, whose length and direction are not altered by an ever changing osseous attachment, are extremely slow to adjust to orthodontic movements of the teeth (Erikson et al., 1945). Edwards (1968) determined that after a lengthy period of retention, up to five months, the fibrous bundles of the periodontal ligament, as well as the transeptal fibres closest to the crest of the alveolar septum, appeared completely adapted to the new rotational position of the tooth. Edwards (1968) observed that this was due to the slow adaptation by the supra-alveolar fibres.

4.5 RELAPSE

Relapse of tooth positions following completion of orthodontic treatment is a major concern, and undesired factor (Bowling and Rygh, 1988). Suggested causes for relapse following orthodontic treatment have included:

i. Poor adaptive tissue reaction in the periodontium (Reitan, 1959).

ii. Compression of supra-alveolar tissue (Erikson et al., 1945; Thompson et al., 1958; Parker, 1972).

iii. The presence of elastic like fibres in the transeptal region (Edwards, 1968; Campbell et al., 1975).

Relapse in orthodontic movement has been attributed to the supra-alveolar structures. Using dogs Reitan (1954) found that after rotation of maxillary lateral incisors, the
supra-alveolar gingival fibres remained stretched and displaced for at least two hundred and thirty two days. However the principal fibres of the periodontal ligament were found to adjust very quickly. It has been shown that gingivectomy and surgical circumferential severing of gingival fibres may reduce relapse (Edwards, 1968).

Brain (1969) orthodontically rotated the maxillary second incisor of five mongrel dogs. Subsequent to the orthodontic rotations, the free gingival fibres attached to the right second incisor were severed; the left incisors which were also orthodontically rotated served as controls. These teeth were retained for at least one hundred and forty eight days. Study casts of tooth positions taken before treatment, before retention, and after retention revealed that the greatest percentage of regression occurred within the first eighteen hours in the control incisors. The stability of the surgical side was evidenced by the fact that there was no measurable relapse in four of the dogs. This project suggested that trans-section of the free supra-alveolar fibres following orthodontic tooth rotation, coupled with a period of retention which was unspecified allowed for optimum biologic repair. This was thought to be a definite adjunct to retention.

Edwards (1968) suggested that oxytalan fibres of an elastic like nature proliferate and accumulate in the gingiva, and particularly in the transeptal ligament during orthodontic tooth movement. Conversely, an experimental model based on orthodontic movement of maxillary molars in rats by Bowling and Rygh (1988) have found oxytalan fibres to have little role in the relapse of orthodontic treatment.

4.6 ROOT RESORPTION

The mineralised tissues of the permanent teeth are not normally resorbed. They are protected in the root canal by predentine, and odontoblasts; and on the root surface by the precementum. If the predentine or precementum become mineralised, or in the case of the precementum is mechanically damaged or scraped off, multinucleated cells will colonise the mineralised or denuded surfaces and resorption will ensue. This type of
resorption has been referred to as inflammatory root resorption. It occurs on the wall of the root canal (internal resorption) and on the external surface of the root (external resorption) and it may be transient or progressive (Tronstad, 1988).

Root resorption occurring during orthodontic treatment seems to be related to local injury of the periodontal ligament and, in particular, to hyalinisation (Rhygh, 1977; Williams, 1984). Careful examination of the root surface of teeth that have been moved orthodontically revealed areas of resorption of both cementum and dentine. These areas tended to fill in with new cementum, so that the original form of the root was maintained (Proffit, 1986). Simon et al. (1981) in a comparison of cellular cementum in healthy and diseased teeth found that there was cementum resorption in all periodontally diseased teeth studied. Batenhorst et al. (1974) stated that some root resorption during orthodontic treatment was unavoidable.

4.6.1 Cervical Root Resorption
Cervical resorption is defined as resorption following injury to an area of the root surface below the epithelial attachment (Brosjo et al., 1990). Teeth with cervical root resorption have often been previously exposed to trauma or orthodontic forces in the cervical area (Brosjo et al., 1990). A study by Brosjo et al. (1990) using a monkey model to determine the aetiology of cervical resorption found that an incomplete epithelial coverage of a cervical marginal dentine surface in combination with marginal gingivitis or periodontitis was sufficient to maintain a progressive cervical resorption. The damaged area of the root surface is colonised by hard tissue resorbing cells. The cervical resorption will usually be transient with cemental repair occurring within two to three weeks without treatment. However stimulation of the hard tissue resorbing cells can be prolonged by bacterial products. The bacterial products gain access via the cervical dentinal tubules, the gingival sulcus, and the surface of the tooth. If the local injuries lead to necrosis of the periodontal ligament tissue in the cervical area, cervical resorption may take the form of ankylosis and replacement resorption (Tronstad, 1988).
4.6.2 Apical Root Resorption

Apical root resorption has been noted to occur following orthodontic treatment, and also subsequently to dental trauma. Linge and Linge (1991) found the following variables to significantly affect apical root resorption during orthodontic treatment:

i. Overjet.

ii. History of trauma to the maxillary incisor teeth before initiation of treatment.

iii. Duration of treatment with rectangular arch wires.

iv. Localised problems, such as impacted teeth.

In post orthodontic cases resorption is often manifested as blunting or shortening of the roots (Solomon et al., 1989). It has been observed that apical root resorption halts upon completion of orthodontic treatment (Tronstad, 1988).

4.7 EXTRUSIVE TOOTH MOVEMENT OF VITAL AND NON VITAL TEETH

Oppenheim (1940) was the first to report the histologic changes that occur during artificial elongation of vital monkey teeth. He reported that extrusion produced stretching of supracrestal and principal fibres which resulted in bone formation at the apex and the alveolar crest of the extruded teeth. Hemley (1953) stated that, after extrusion of a tooth a layer of osteoid formed along the sides of the alveolus, and unless properly controlled both bone and cementum in the apical region were resorbed. Hirschfield and Geiger (1966) stated that vital root elongation could be accomplished easily because only the periodontal fibres opposed any vertical movement, and little if any bone resorption occurred. Reitan (1967) reported, from clinical and histological studies on human and animals after orthodontic movement that the principal fibres of the periodontal ligament rearranged position and return to their normal alignment quickly during retention, whereas the supracrestal fibres may remain stretched for long periods. Reitan (1967) subsequently felt that vital extrusion should not exceed forces of twenty five to thirty grams and should be accomplished in seven to eight weeks.
A histological study by Mostafa et al. (1991) was performed on 36 intact maxillary first premolars of young adult orthodontic subjects. The maxillary first premolars were extruded under controlled conditions with the aid of fixed edgewise orthodontic appliances for either one, two, or four weeks. These authors reported the following histological effects on the dental pulp of the premolar teeth:

i. Circulatory disturbances with congested and dilated blood vessels.

ii. Odontoblastic degeneration, vacuolisation of the pulpal tissues.

iii. Fibrotic changes.

They felt that odontoblastic degeneration was most probably the result of a compromised blood supply.

Batenhorst et al. (1974) performed a study on two adult rhesus monkeys to examine tissue changes resulting from facial tipping and extrusion of incisors. These authors placed orthodontic bands on the six mandibular incisor teeth. The left central and lateral incisors underwent labial tipping and extrusion (approximately 5mm), while the contralateral central and lateral incisor teeth acted as controls. The period of activation was fifty four days for one monkey and sixty four days for the other monkey. They reported that the width of the attached gingiva increased on the labial aspects of all the experimental teeth but remained the same over the control teeth. Gross examination of the defleshed mandible revealed a dramatic build up of bone on the lingual and interproximal surfaces of the experimental teeth. Histologically, the authors observed that the supracrestal fibres on the buccal surfaces of the experimental teeth paralleled the root surfaces rather than inserting at right angles into the cementum. Just apical to the epithelial attachment on each experimental tooth was an area of root resorption, which extended into dentine in some sections and was lined with a new layer of cementum. The most dramatic finding of their study was the extensive bone apposition that occurred on the mesial, distal, and lingual surfaces as the teeth extruded. They also found clinically the presence of moderate gingivitis following the placement of the orthodontic bands.
In recent times, work has been carried out on the effects of short term, continuous extrusive forces on the vascular bed of the periodontal ligament of vital teeth. Picton and Moss (1984) performed a study on short term extrusion of isolated teeth in adult monkeys. Twelve teeth from six adult male monkeys were studied. A transducer was attached to the occlusal surface of the test tooth by means of impression compound and cyanoacrylate cement. A dentine screw was inserted into the alveolar process approximately 1cm from the test tooth. This device was a variance capacitor used to electronically measure the amount of extrusion of the experimental tooth. The authors found that in a 40 to 360 minute period, extrusion of these vital teeth varied from 10μm to 138 μm. They felt that these tooth movements were caused by forces generated in the periodontal ligament resulting from the biochemical changes in the periodontium rather than by vascular alterations.

Lew et al. (1989) applied a continuous load of 1.0N to the maxillary first molar of a rat for thirty minutes. Their extrusion device consisted of modified tweezer beaks with a quick release device. They found statistically significant changes in the microvascular bed of the tensioned periapical ligament. Their study was a transmission electron microscope analysis. They found that the mean vascular volume, as a percentage of periapical ligament volume increased in post capillary sized venules (from 16.6% to 22.3%), venous capillaries (from 2.0% to 2.7%), arterial capillaries (from 0.4% to 1.0%), and terminal arterioles (from from 1.0% to 2.5%).

Using the extrusion device of Lew et al. (1989), Cooper and Sims (1989) examined the effect of a continuous load of 100 grams applied to the right maxillary first molar of eight rats for thirty minutes. The maxillary left molar served as a control. An ionic tracer was used to mark the water rich channels of the extravascular tissues. The study observed the presence of early signs of acute inflammation. Red blood cell diapedesis was observed. This is an early sign of the inflammatory process. From this study the
authors concluded that "light appliance activation compatible with cell stimulation and survival should be used in orthodontic tooth movement".

Crawford et al. (1986) in a study on unopposed rat molars found a significant increase in extrusion of an unopposed molar. Mandibular molars of five male rats were extracted and it was found that the lack of centric stops led to the extrusion of the maxillary molar into the extraction site. The animals were euthanised after three days and the changes associated with the periodontal ligament of the extruded maxillary molar were examined. They found a trend for decreased periodontal ligament width of the extruded molar and no significant difference in total cells, cell density or relative distribution of fibroblast like cells. The unopposed molar was also found to retain good osteogenic potential.

Steadman (1942) stated that endodontically treated teeth which were orthodontically moved were likely to have their root "melt away". This was thought to be due to the endodontically treated tooth acting as a foreign object, and causing a chronic irritation. Heuttner and Young (1955) in a study on monkeys refuted this statement, and concluded that no difference existed between normal and endodontically treated teeth. They observed apical bone resorption but did not observe bone deposition at the alveolar crest.

A clinical study was carried out by Spurrier et al. (1990) to determine whether both vital and endodontically treated teeth, exhibited a similar severity of apical resorption in response to orthodontic treatment. Forty three human patients who had one or more endodontically treated incisors before orthodontic treatment were studied. In each patient the vital contralateral tooth served as a control. Radiographs were taken of the experimental and control teeth. They found that vital incisors resorbed to a significantly greater degree than endodontically treated incisors (p ≤ 0.05 ). Even though statistical differences were reported, clinical differences were reported to be minimal.
Simon et al. (1980) extruded endodontically treated decoronated premolar teeth in three dogs. Special chrome cobalt bars were cast to approximate canine-molar length. This bar was inserted into proximal slots prepared in the canines and molars. To aid in retention of the bars, pins were placed in buccal or lingual tooth structure and reinforced by placing acid etched composite resin over the bar, pins, and slots. The appliance was activated by placing an orthodontic elastic from the cobalt bar to the teeth undergoing extrusion. Observations were kept over either a two, four, or seven week period. The authors noted that the alveolar housing was found to move occlusally with the extruded tooth. Bone deposition at the alveolar crest and throughout the interradicular portion, was observed seven weeks after extrusion was commenced. The main limitations to this study as listed by Simon et al. (1980) were;

i. The exact amount of extrusion of each tooth was not measured.
ii. The extrusive force per tooth was variable and not measured.
iii. The period taken to extrude the teeth was not recorded, but in every case it was less than one week.

Ingber (1976a) in a clinical study of moving endodontically treated teeth occlusally, also noted movement of the alveolar crest with the extruded tooth. A histological study of forced eruption in monkeys by Melsen (1986) also demonstrated that the bone produced by forced eruption was maintained.

Simon et al. (1980) declared that extrusion of endodontically treated teeth presented no apparent histological or clinical problems. Radiolucent areas that appeared during the extrusion period were resolved by the fourth week. Also there was no unequal osteoid formation or tearing of the periodontal ligament fibres. Interestingly the midpoint of the periodontal ligament appeared disjointed, but the fibre attachments to bone and cementum remained vital. By the seventh week the histological appearance was normal with the epithelial attachment remaining at the cementoenamel junction and the distance from the epithelial attachment to the crestal bone appearing normal. The periodontal
ligament fibres appeared more horizontally placed than before but looked essentially normal. Histologically, at seven weeks, new bone was formed at the alveolar crest, interradicular, and apical areas. The crestal bone was composed of mature bone and was similar to the controls. The authors considered that the periodontal ligament responded to the extrusive movement by reorientating the periodontal ligament fibres in a normal direction. The periodontal ligament appeared normal after seven weeks. These authors also reported no large areas of resorption or nonattachment of the periodontal ligament. Simon et al. (1980) concluded by noting that the extrusion of endodontically treated premolars in dogs was acceptable.

Steigman et al. (1987) performed a study on the structural changes in the dental and periodontal tissues of the rat incisor following application of orthodontic loads. Their investigation was carried out on forty five young adult female rats, ten of these rats acted as the control group. Continuous linguointrusive loads were applied to the shortened incisor teeth of the experimental animals for a two week period by means of a closed coiled spring. After this period five rats were euthanised with the spring in situ, in the remaining animals the springs were removed and the animals were euthanised in groups of five, at one, three, five, seven, nine, or ten weeks. They found that as a consequence of loading the total periodontal ligament volume increased by 72%. There was an even greater change at the apical third of the tooth, an addition of 177% to the periodontal ligament volume. After removal of the springs the periodontal ligament volume was found to return to normal. At the end of the experiment the expanded periodontal ligament volume was larger by 50% than that of normally erupting teeth.

Crowe (1989) examined the extrusion of the left central incisor in eight cotton-eared marmosets. The incisor teeth in the marmosets were only 6mm long. After decoronation with a high speed bur and completion of a root canal therapy the animals underwent two weeks of extrusive movement. The teeth were held in retention for a further nine weeks. A transmission electron analysis was performed on the extruded
teeth. Crowe (1989) found changes related to the vascular and neural supply of the extruded teeth.

Weir and Sims (1991), using marmosets in a follow up study to that of Crowe (1989) extruded the maxillary central incisor in four of these animals following root canal therapy. A samarium-cobalt magnet was cemented into the decoronated incisor root surfaces, and individually cast cobalt-chrome devices bonded to the maxillary anterior teeth with an identical magnet fixed in a housing over the treated incisor. Each maxillary incisor was extruded 1.2mm and retained for thirty weeks. Tissue blocks were prepared for TEM analysis. Their analysis revealed there were no significant morphological differences between the control and experimental microvascular beds following retention. However they found extrusion and long term retention was accompanied by reconstitution of the periodontal ligament vascular and neural systems.

In summary the biologic effects of orthodontic tooth movement are complex. It appears that tooth movement is related to the concept of undermining resorption. The movement of a tooth and the alterations to the periodontal ligament and its cellular elements appear to be related to the blood flow within the periodontal ligament. The most acceptable theory of orthodontic tooth movement is the "blood flow theory" such that alterations in local vasculature lead to progressive tooth movement. Orthodontic extrusion of vital and non vital teeth leads to alterations in the alveolar bone height, cementum deposition, fibres of the periodontal ligament and to the vascularity of the periodontal ligament.
5 MATERIALS AND METHODS

5.1 THE EXPERIMENTAL ANIMAL

The extrapolation of experimental results using animal models to humans is always subject to much debate. The ethical issues related to animal research have further widened this controversial issue. Barker (1970) evaluated the feasibility of using dogs for experimental research and the extrapolation of results to the human subject. He concluded that the periapical tissues in the dog and the human do react in a comparable manner to endodontic treatment. Speelman and Collaert (1990) have shown beagle dogs to be successfully utilised for dental research, in particular periodontal disease.

In this study beagle dogs were chosen as the most suitable experimental animal by virtue of:

i. Size, as beagle dogs can be handled relatively easily.

ii. Their incisor morphology, as the pulp chamber and size of the teeth are easily accessible for endodontic treatment.

iii. Uniform genetic make up.

iv. Cost.

This study began with ten beagle dogs obtained from a colony bred at Meadow Mist Kennels in Sydney, New South Wales. The animals were housed within the animal vivarium of Westmead Hospital throughout the experimental treatment period. Three of the animals were discontinued due to repeated appliance breakage and animal fighting (table 5-1). In total, five beagle dogs acted as experimental animals and the remaining two beagle dogs acted as control animals. The animals were vaccinated against Hepatitus, Distemper, and Parvovirus. The experiments were performed according to
the National Health and Medical Research Council\(^1\) statement on animal experimentation which provided guideline's for the care and use of animals for research purposes, and also according to the guideline's for animal research outlined by the Animal Ethics Committee of Westmead Hospital.

<table>
<thead>
<tr>
<th>BEAGLE(^2)</th>
<th>AGE (MONTHS)</th>
<th>VACCINATION(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>hep, dis, parv</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>hep, dis, parv</td>
</tr>
<tr>
<td>C</td>
<td>11</td>
<td>hep, dis, parv</td>
</tr>
<tr>
<td>D</td>
<td>discontinued</td>
<td>hep, dis, parv</td>
</tr>
<tr>
<td>E</td>
<td>11</td>
<td>hep, dis, parv</td>
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<td>hep, dis, parv</td>
</tr>
<tr>
<td>G</td>
<td>discontinued</td>
<td>hep, dis, parv</td>
</tr>
<tr>
<td>H</td>
<td>discontinued</td>
<td>hep, dis, parv</td>
</tr>
<tr>
<td>I</td>
<td>11</td>
<td>hep, dis, parv</td>
</tr>
<tr>
<td>J</td>
<td>9</td>
<td>hep, dis, parv</td>
</tr>
</tbody>
</table>

Table 5.1 Details of the experimental animals.

5.1.1 Oral Hygiene Procedures

The beagle dogs were housed together prior to the initiation of dental treatment. Upon commencement of treatment the animals were separated into individual cages to decrease the incidence of fighting. The animals had their teeth brushed regularly, using a small Oral B\(^4\) toothbrush dipped in 0.2% chlorhexidine\(^5\) gel. This regime was performed throughout the entire treatment period.

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\(^1\)Australian code of practice for the care and use of animals for scientific purposes. NHMRC, 1990.
\(^2\)For ease of description animals A, B, C, E, F, I, and J will be known as 1, 2, 3, 4, 5, 6, and 7 respectively for the remainder of this text.
\(^3\)hepatitis, distemper, parvovirus.
\(^4\)Oral B Lab, Pty Ltd. Sydney, Australia
\(^5\)Chlorhex Gel. Wallace Oropharm. Melbourne, Vic.
5.1.2 Dietary Requirements

Prior to the initiation of treatment the animals were fed once daily with a combination of Pal Meatybits and Pal canned dog food\(^1\), or beef mincemeat. After initiation of treatment the animals were fed a combination of Pal canned dog food and beef mincemeat only. This was performed in an attempt to reduce the incidence of trauma and damage to the orthodontic appliances resulting from the animals active chewing habits and mastication of hard food.

5.2 THE EXPERIMENTAL PROCEDURE

Dental procedures were carried out under general anaesthesia in the operating theatres of the animal vivarium. Qualified veterinary surgeons and veterinary attendants were available for assistance and consultation throughout each operating session.

5.2.1 General Anaesthesia

The animals were treated under general anaesthesia via the following protocol:

i. The beagle dogs were weighed.

ii. Acetyl Promazine (0.1mg/kg)\(^2\) premedication was given intramuscularly with a 13mm 23 gauge needle\(^3\) thirty minutes prior to oral intubation. Each animal was firmly held by an attendant and the premedication was given intramuscularly in the right hind leg. After the animal began to show signs of drowsiness and loss of balance it was prepared for intubation.

iii. Each of the animals had their right foreleg shaved to expose a long saphenous vein which was used for cannulation. A 23 gauge 19mm

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\(^1\)Pal Wodonga, Victoria, Australia.
\(^2\)Acetyl Promazine Maleate 2mg/ml.
\(^3\)Terumo. Melbourne, Australia.
butterfly needle\textsuperscript{1} was used to administer the anaesthetic agent, Pentothal (5g/100mL)\textsuperscript{2} intravenously

iv. Once the animal was anaesthetised a size 7.0 Rae Endotracheal Tube\textsuperscript{3} was passed down the airway with the aid of a straight laryngoscope\textsuperscript{4}. The animals were then maintained on 2\% gaseous Fluothane\textsuperscript{5} anaesthesia with 1L oxygen, and 3L nitrous oxide, via a Midget 3\textsuperscript{6} anaesthetic machine. Each animal was placed in a supine position. The anaesthetic tube was secured to the mandible by cotton ligatures. The level of the gases was altered depending upon the degree of consciousness of the animal during the procedure.

v. Throughout the procedure meticulously monitored were the animals:

i. Body temperature.

ii. Muscle tone.

iii. Eye movements.

vi. The animals were firmly secured throughout each general anaesthetic procedure to the operating table by leg ropes. A body warmer was placed on the operating table to ensure that no abnormal decreases in body temperature occurred. The mouth was kept open with the use of a mouth prop.

vii. Upon completion of the treatment each animal was extubated and placed in the recovery bay. The beagle dogs usually took at least thirty minutes to recover from the effects of the anaesthetic. They were not placed in the recovery room until they showed signs of consciousness.

\textsuperscript{1}Surflo Winged infusion set, Terumo. Melbourne, Australia.
\textsuperscript{2}Pentothal (Thiopentone sodium BP), veterinary anaesthesia, Boechnering Ingleheim.
\textsuperscript{3}Rae endotracheal tube size 7.0. Mallinkrodt Lab. Athlone, England
\textsuperscript{4}Welch Allyn 2 straight laryngoscope.
\textsuperscript{5}Halothane, ICI. Melbourne, Australia.
\textsuperscript{6}Midget 3 anaesthetic machine. CIG, Australia.
5.2.2 The Experimental and Control Teeth

The maxillary teeth were chosen in preference to the mandibular teeth, to decrease occlusal interference. The upper right first lateral incisor tooth was chosen to be the experimental tooth. It was chosen for its ease of access and manipulation during treatment, also there were sufficient teeth on either side of it to provide anchorage during orthodontic extrusion. Also the central incisor was situated next to a fibrous midline suture which may have influenced observation of the periodontal ligament. After anaesthetising the animal the crown of the maxillary right first lateral incisor was decoronated with a high speed diamond bur just coronal to the gingival margin (figure 5-1, figure 5-2).

The control teeth in animals six and seven underwent the same protocol as the experimental teeth. After endodontic treatment and crown decoronation these two animals were left untreated until sacrifice. Orthodontic appliances were not placed on the two control animals.

5.2.3 Root Canal Therapy

Following decoronation of the upper right first lateral incisor a vital pulpectomy was performed on this tooth. A conventional lateral condensation root canal therapy was completed (table 5-2). Appropriate radiographs\(^1\) were taken and the tooth was obturated with Gutta Percha\(^2\) master points and fine-fine accessory points, with Procosol\(^3\) root canal sealing cement.

\(^1\)Periapical radiographs with Kodak fast speed film.
\(^2\)Progress, hand rolled gutta percha points. Rudolf Gunz, Sydney.
\(^3\)Procosol Root Canal Sealing Cement, Dentalez Inc. Lancaster, Pa.
Figure 5-1  The decoronated upper right first lateral incisor of a beagle dog.

Figure 5-2  Palatal view illustrating the decoronated lateral incisor tooth.
<table>
<thead>
<tr>
<th>Beagle</th>
<th>Root canal length (mm)</th>
<th>Master point size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.5</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>15.0</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
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<td>40</td>
</tr>
<tr>
<td>7</td>
<td>14.5</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 5-2. Details of root canal therapy for the individual beagle dogs.

5.2.4 The Extrusion Device

After completion of root canal therapy on each experimental tooth, the root canal was prepared for post placement. A stainless steel posted was then cemented into the root canal with zinc phosphate cement\(^1\) (figure 5-3). Beddtiot\(^2\) orthodontic brackets were cemented to the six maxillary anterior teeth, from the right second lateral incisor to the left second lateral incisor with Ormco System 1\(^3\) cement. A 0.012 inch archwire\(^4\) was then positioned with an appropriately shaped loop for attachment to the stainless steel post in the experimental tooth. The archwire was then ligated to the stainless steel post. Power O rings\(^5\) were used to secure the archwire into the orthodontic brackets (figure 5-4, figure 5-5).

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\(^1\)SS White Pennwalt. Harrow, Middlesex.
\(^2\)Beddtiot orthodontic brackets. JT Jennings, Sydney.
\(^3\)System 1 orthodontic bonding adhesive, Ormco. Glendora, California.
\(^4\)AJ Wilcock light wire, 0.012 inch stainless steel (special plus). Whittlesea, Victoria.
\(^5\)Ormolastral power O rings, Ormco. Glendora, California.
Figure 5-3  Stainless steel post cemented into root filled lateral incisor of animal one.

Figure 5-4  Labial view of the archwire ligated to the stainless steel post in the experimental tooth of animal one.
5.2.5 The Extrusion Protocol

In the five experimental animals the maxillary right first lateral incisor underwent a specified protocol of orthodontic extrusion. Initial extrusive forces ranging from 30 to 120 grams were applied to the lateral incisor. The specific force was measured with a Correx\textsuperscript{1} strain gauge. The control teeth did not undergo extrusion.

\textsuperscript{1}Correx strain gauge. Haggstreit, Switzerland.
The orthodontic extrusion protocol was as follows (table 5-3):

i. **Extrusive force:**
   
   This force varied from 30 to 120 grams. For the first four experimental animals an attempt was made to have two animals undergo extrusion with light forces and two animals undergo extrusion with heavy forces. It was planned to pair the specimens for comparison under the scanning electron microscope and the light microscope.

ii. **Post Extrusive Management:**

   Of the five experimental animals four were subjected to:
   
   a. A retention period following the active extrusion phase.
   b. A waiting period before sacrifice.
   c. One animal (number 5) had no retention period, but had a periodontal surgery procedure in lieu of retention followed by a waiting period prior to sacrifice.

The experimental animals consequently underwent four to six weeks of active extrusion. During that period of time the animals suffered appliance breakages which on occasions necessitated a general anaesthetic to reposition the archwire. Three of the original ten animals were removed from the study due to repeated appliance breakage.
<table>
<thead>
<tr>
<th>Beagle (number)</th>
<th>Initial Extrusive force (grams)</th>
<th>Extrusion period (weeks)</th>
<th>Retention period (weeks)</th>
<th>Waiting period (weeks)</th>
<th>Total treatment (weeks)</th>
<th>Analysis (SEM/LM)</th>
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<tr>
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<td>None</td>
<td></td>
<td></td>
<td>40</td>
<td>SEM</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>None</td>
<td></td>
<td></td>
<td>40</td>
<td>LM</td>
</tr>
</tbody>
</table>

Table 5-3  Experimental regime for the beagle dogs.

5.2.6  Retention

On completion of the active extrusion phase the extruded teeth were retained for approximately twelve weeks for all of the animals with the exception of animal five and the two control animals. Retention was achieved by leaving the appliances in position and utilising a passively positioned archwire.

5.2.7  Waiting Period

Upon completion of the retention period, the animals were debanded. The animals were left debanded for a further eighteen to twenty weeks to determine if relapse had occurred. This was the waiting period.

5.2.8  The Periodontal Surgical Procedure

Animal number five underwent a periodontal surgical procedure in lieu of retention to determine whether the effects of severing the supra-alveolar fibres prevented relapse of

¹Beagle no. 5 had a periodontal surgery procedure in lieu of retention, consequently its treatment period was considerably less than the other animals.
the orthodontically moved tooth. After the active extrusion period the animal was then anaesthetised. The orthodontic brackets were removed. A three sided mucoperiosteal labial flap was raised, and at the same time the supra-alveolar fibre attachments were severed. A palatal flap was also raised and the supra-alveolar fibres severed. Upon completion of the periodontal surgical procedure, the soft tissues were sutured to their original positions with 3/0 catgut\(^1\). This animal had no further treatment until sacrifice (figure 5-6 to 5-8).

![Image of periodontal surgical procedure](image)

**Figure 5-6** Photograph illustrating the labial flap of the periodontal surgical procedure performed in animal number five.

\(^1\)3/0 catgut. B Braun Australia Pty Ltd.
Figure 5-7  Photograph illustrating the palatal flap of the periodontal surgical procedure in animal five.

Figure 5-8  Completion of periodontal surgical procedure in animal number five.
5.2.9 Records

Standardised records in the form of colour slides, periapical radiographs, and study models were taken at the following stages of treatment:

i. Prior to endodontic treatment and orthodontic band placement.

ii. At each adjustment stage (ie at the commencement of retention, and at the commencement of the waiting period following debanding).

iii. At the completion of treatment.

Slide photography:
The slide photography was taken with a 35mm SLR camera\(^1\) using Kodachrome 64 colour film\(^2\).

Radiographs:
Periapical radiographs were taken with a Siemens Heliodent Mobile X Ray unit\(^3\). Radiographs were taken during the endodontic procedure and at the specified intervals as previously listed.

Prior to the radiographs being, taken a Formasil\(^4\) key was constructed using the maxillary teeth as landmarks. The was to enable the placement of each periapical radiograph in the same position.

Study Models:
Impressions of the maxillary teeth were taken with an alginate impression paste\(^5\). A stock plastic impression tray\(^6\) which was cut to size to fit the maxillary teeth was used to perform the impressions. The impressions were

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\(^1\)Minolta autobellows III with a 100mm lens and a super ring flash

\(^2\)Kodachrome 64, 35mm colour slide film. Kodak, Australia.

\(^3\)Siemens Heliodent mobile X-ray unit, 70Kvp, 7ma. Germany.

\(^4\)Formasil, silicon based impression material. Kulzer, West Germany.

\(^5\)Jeltrate alginate impression material type I fast set, Caulk Dentsply. Milford, Delaware.

\(^6\)Coe plastic impression tray. Dentsply
then poured up with Type 1 Dental stone\(^1\). These study models were utilised to examine the amount of extrusion or relapse, and the gingival contour of the teeth.

5.3 SACRIFICE

At the completion of the experimental period the animals were sacrificed via the following procedures.

5.3.1 Surgical Procedure

A longitudinal subdermal dissection, 4 to 5 cm in length, was made bilaterally in the anterior part of the neck. The overlying fascia was removed via a blunt dissection technique. The carotid sheath structures on both sides of the neck were located deep to the sternohyoid and omohyoid muscles and lateral to the trachea. Using a pair of fine dissecting scissors, the common carotid artery, the external jugular vein and the vagus nerve on both sides of the neck were located and separated. The overlying fascia was stripped from these vessels and two loose 4/0 silk sutures were passed underneath each artery and vein.

The distal sutures of the artery were then held by one operator to keep the vessel exposed and to stem blood flow. A small incision was made in the artery wall with a fine pair of dissecting scissors. The arteries on both sides of the neck were then cannulated with a pre-prepared silastic perfusion tube attached to a perfusion bottle. The external jugular veins were cannulated in the same manner except the tubing led to a collection bottle to drain the blood and the washout solution. The tubing was securely tied in place with the upper suture, and the caudal part of the vessel was tied off using the remaining suture (figure 5-9).

\(^1\)Velmix dental stone, Kerr. Michigan, USA.
Figure 5.9  Bilateral neck dissection illustrating the neck vessels located, with sutures placed around them. Blood vessels (BV), sutures (SUT).

5.3.2  Perfusion

Prior to perfusion fixation, a vascular wash out was performed via the carotid arteries (Appendix 1). 1.5L of a heparinised saline solution was introduced at a rate of 300ml/min. with a peristaltic pump\(^1\) until a clear exudate flowed from the drainage tube. Once the exudate was clear, the fixation solution was introduced by turning a three way valve to allow inflow of the perfusion solution.

The tissues were then fixed by vascular perfusion with paraformaldehyde in a phosphate buffer solution (Appendix 2). This solution was delivered at a rate of 300ml/min. 1.5L of fixative was delivered to each animal over a five to ten minute period or until the animal showed signs of fixation.

\(^1\)Gambro peristaltic pump, Ab Instrumenta. Lund, Switzerland.
The head and neck was considered well perfused when the following signs were present:

i. The jaws were blanched and rigid.

ii. The tissues of the jaw became firm and had lost their warmth and texture.

At the completion of the fixation procedure an intravenous solution of Apex Euthanasia solution\(^1\) was given to the animal.

5.3.3 Dissection

Following perfusion the animals had the anterior regions of their maxillary jaw removed. Using a sharp handsaw the anterior maxilla from canine to canine was removed. The specimen blocks were then placed into a solution of paraformaldehyde based fixative until processing procedures could be initiated.

5.4 LIGHT MICROSCOPY

Before processing was initiated the premaxillary blocks were trimmed with a scalpel so that the region to be decalcified included the experimental tooth and the second lateral incisor.

5.4.1 Decalcification

The appropriate sections were placed into labelled glass beakers containing a 10% EDTA solution (ph 7.4). Solution changes were carried out every two days until decalcification was complete. This was approximately three to four months in duration. The end point of decalcification was determined radiographically, and by probing the specimen with a dental probe in an unwanted region (figures 5-10 to 5-11).

\(^1\)Sodium Pentobarbitone BP in stabilised solution, Apex Lab Pty Ltd. St Marys, Australia.
Figure 5-10  Radiographic appearance of tissue block of animal two prior to decalcification. Gutta percha (GP) present, and adjacent calcified tissue (CAL).

Figure 5-11  Radiographic appearance of tissue block of animal three following decalcification.
5.4.2 Tissue Processing

Upon the completion of the decalcification procedure the tissues were dehydrated and prepared for infiltration and embedding. Using a sharp scalpel the experimental tooth and the periodontium was sectioned sagitally in the midline. One half was then prepared for horizontal cross sectioning and the other half for buccal palatal vertical sectioning (figure 5-12). The tissue processing procedure was as follows:

i. Wash

The tissues were washed with distilled water and then left in this solution for 30 minutes.

ii. Dehydration

2 x 60 minutes in 50% alcohol
2 x 60 minutes in 70% alcohol
2 x 60 minutes in 80% alcohol
2 x 60 minutes in 90% alcohol
2 x 60 minutes in absolute alcohol

iii. Infiltration

1 x 60 minutes LR White¹ and absolute alcohol (a ratio of 1:1)
1 x 60 minutes LR White
Left in LR White overnight to complete infiltration

iv. Embedding and infiltration

The specimens were placed in rubber moulds filled with the resin. The moulds were then placed into an oven and the resin polymerised over 18-24 hours at 60°C

Figure 5-12 Plane of sectioning of specimens for histological examination. Tooth to the right is root filled decoronated tooth, tooth to the left is the non root filled second lateral incisor. 1. Buccal view. 2. Coronal view. A: indicates that the tooth was divided in half vertically; B: Longitudinal serial sections were cut bucco-lingually at 5μm intervals from one half; C: Horizontal sections were cut from the other half at 5μm intervals. The boxed region in the coronal view illustrates the area used for histological analysis.

5.4.3 Tissue Sectioning

The polymerised blocks were trimmed with a scalpel and reduced in size and secured in a Leitz 1516¹ microtome.

¹Leitz 1516 microtome. North Ryde, Australia.
Using a tungsten carbide knife\textsuperscript{1} serial sections were cut at 5\textmu m intervals. The sections were then transferred to a hot water bath\textsuperscript{2} at a temperature of 60\degree C. The sections were flattened and transferred to a glass slide. The glass slide and specimen were then placed onto a hot plate\textsuperscript{3} until moisture was eliminated from the specimen. Tissue sectioning was completed in this manner for both vertical and horizontal sections.

5.4.4 Tissue Staining

After sectioning and drying the histological sections were then stained with Haematoxylin and Eosin via the following procedure:

\begin{enumerate}
  \item Distilled water wash.
  \item Sections stained with Mayers Haematoxylin for 30 minutes.
  \item The section was transferred after a short time in distilled water, into running tap water to "blue" the haematoxylin stained tissue.
  \item After washing the section was then counter stained with a 5\% solution of Eosin Y in distilled water.
  \item Brief wash in distilled water. The sections were then dried and mounted in DPX\textsuperscript{4}.
\end{enumerate}

Selected sections were stained with Tri-chrome stain following the above method. The sections were then coded and stored in slide boxes.

5.4.5 Light Microscopy Analysis

Light microscopy examination was performed with the aid of an Olympus Vanox microscope\textsuperscript{5}. Horizontal and vertical sections were examined.

\textsuperscript{1}Tungsten Carbide knife, Leitz. North Ryde, Australia.
\textsuperscript{2}Teledyne Hanan hot water bath. Buffalo, NY.
\textsuperscript{3}Corning Hot plate PC 101. Corning Glass Works, NY.
\textsuperscript{4}DePex Mounting Medium, BDH Chemicals Ltd. Poole, England.
\textsuperscript{5}Olympus Vanox AHB-CB, Selby Anax. Lidcombe, Sydney.
i. **Horizontal Sections**

Each tooth was divided into three equal regions, cervical, mid-root, and apical. One histological section from each region was selected for examination. An examination of the interproximal region between the second lateral incisor and the experimental tooth only was performed.

ii. **Vertical Sections**

The most well stained and intact vertical sections were examined and analysed.

Photographs of the specimens were taken with a Olympus Vanox photography unit and a 35mm camera attached to the microscope. Ilford Pan F Black and White film, Kodacolour 100, and Kodak Ektachrome colour film was used. Photography was developed commercially.

### 5.5 SCANNING ELECTRON MICROSCOPY

After block dissection of the specimens they were prepared for scanning electron microscope analysis. The extruded tooth and the surrounding alveolus was sectioned sagitally with a diamond disc. From one half of the tissue block the tooth was extracted and the root surface rendered anorganic with 5% sodium hypochloride. After air drying the specimen was critical point dried with a Balzers Critical Point Dryer\(^1\) and gold coated with a Balzers Sputter Coater\(^2\).

The other half of the tissue block was fixed for seven days in paraformaldehyde and then demineralised in 10% EDTA (ph 7.4) as noted for the light microscope section. This tissue block was sectioned in half mesio-distally. From these two halves one block was sectioned horizontally at 2mm intervals, and the other half was sectioned vertically in a bucco lingual orientation at 2mm intervals (figure 5-13). The sections

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1. Balzers Union. Germany.
2. SCD 030. Balzers Union, Germany.
were then washed in distilled water, dehydrated through a graded ethanol series, critical point dried and gold coated. The specimens were coded prior to storage.

Figure 5-13  Plane of sectioning of specimens for SEM analysis. Extruded tooth is on the right. The tooth was divided vertically. Portion A was rendered anorganic and this half was examined in toto. The remaining half was decalcified and divided mesial-distally, as illustrated by the arrow in the coronal view. Portion B was sectioned vertically, and the boxed area in the buccal view illustrates the region examined. Portion C was sectioned horizontally at 2mm intervals, as illustrated by the dotted lines in the buccal view. The boxed area in the coronal view illustrates the regions examined horizontally in portion C.
5.5.1 Scanning Electron Microscope Analysis

Scanning electron microscopy was performed with a Jeol 840 Scanning electron microscope\textsuperscript{1}, at 15KV accelerating voltage. Specimens were examined and photographs were taken. A Mamiya 35mm camera attached to the scanning electron microscope was used to record the photographs, Ilford FP4 black and white film was used. All horizontal and vertical sections had photographs taken at a magnification of 40 and 140. At higher magnifications a montage was necessary to include the entire tooth structure.

The film was developed using Ilford ID2 and Ilford Hypam rapid fixer according to manufacturers instructions. The negatives were printed onto Kodak Polycontrast III RC black and white paper using a Durst RCD 3400 enlarger\textsuperscript{2}. The print was developed in Ilfospeed paper developer and fixed with Ilfospeed paper fixer\textsuperscript{3}.

Stereo pair photographic examination of all SEM sections was also performed. Photographs of selected areas were taken and the microscope was tilted through 60°. The area just photographed was centred on the screen and refocused with the Z control. Examination of these sections was performed with a stereo microscope\textsuperscript{4}.

5.6 GENERAL ANALYSIS

Light microscope examination of histological slides concentrated on observing any cellular changes when compared with the control animals. The SEM observations highlighted the reactions of the fibrillar elements of the ligament to the extrusive movement. Areas of cementum resorption and deposition were also identified in the SEM and histological sections.

\textsuperscript{1}JSM 840, Jeol. Japan.
\textsuperscript{2}Durst, West Germany.
\textsuperscript{3}Ilford Aust Pty Ltd, Victoria.
\textsuperscript{4}Wild Heerbrug stereomicroscope. Heerbrug, Switzerland.
5.6.1 Clinical Appearance and Measurement of Extrusion

The clinical photographs taken were used to monitor the health of the gingival tissues and the effects of the orthodontic appliances upon the soft tissues. The amount of extrusion was determined with the aid of the study models. A novel way to determine the relative extrusion of the experimental teeth to the adjacent teeth was developed. As implanted markers were not positioned the only means of determining movement of the experimental tooth was to assess the position of the experimental tooth from the study models. The study models for all of the animals were surveyed with a dental surveyor. Utilising tripod marks made by the dental surveyor the study models were orientated in the same horizontal and vertical plane to each other. They were then trimmed with a model trimmer. A small piece of brass wire (0.5mm) was placed on the experimental tooth and one tooth on either side of this experimental tooth (figure 5-14). Radiographs of these brass points were taken with a paralleling technique and in this way it was possible to measure the amount of extrusion of the experimental tooth in relation to the adjacent teeth (figure 5-15). A series of measurements were obtained at each stage of treatment and it was possible to determine the alterations in extrusion and relapse, and movement of the gingival margin.

![Figure 5-14](image)

**Figure 5-14** Brass points in position on study model. Model prepared for radiographs, and measurement of the changes in gingival margin height and the amount of extrusion and relapse of the decoronated tooth.
Measurements of the amount of extrusion and gingival margin movement were made from a line drawn perpendicular to the line joining the central incisor and the second lateral incisor. Measurements were made with vernier callipers (Appendix 3).

![Figure 5-15](image)

**Figure 5-15** Radiograph of study model of animal one prior to extrusion. Brass point (BP), central incisor (CI), experimental tooth (EP), second lateral incisor (LI), gingival margin (GM).

### 5.6.2 Radiographic Review

Radiographs taken at the specified intervals previously listed were used to determine the success of the root fillings and to determine the success or failure of the extrusion technique.

### 5.6.3 Quantitative Analysis

In this initial study using a small sample size it was not possible to determine if any significant difference was present between the various animals. To determine trends present in the experimental procedure an examination of the interproximal region between the extruded tooth and the adjacent second maxillary right lateral incisor was made. The parameters examined were:

i. Mean vascular area of blood vessels.

ii. The number of blood vessels.
iii. The mean diameter of the blood vessels.
iv. The mean thickness of the cementum.
v. The mean thickness of the periodontal ligament in the selected interproximal region.

In determining the size of the blood vessels and the corresponding widths of the cementum and periodontal ligament a computer analysis of the selected regions was performed. Three horizontal sections from each tooth (control and experimental) were selected to represent the cervical, mid-root, and apical regions of the tooth. With photographs at a magnification of X140 a specified area of the interproximal periodontal ligament was measured (210x140µm). This area was uniform for every section examined. The Bioquant programme\(^1\) linked to a NEC computer\(^2\) and a digitizing pad\(^3\) was used to measure the parameters described above (figure 5-16).

Measurements of the above parameters were performed in the interproximal region between the root filled tooth and the maxillary right second lateral incisor, in this manner a direct comparison could be made between the root filled and non root filled tooth (figure 5-17).

\(^1\)BQ System IV. RAM Biometrics Inc.
\(^2\)Power Mate 386/215. NEC, Japan.
\(^3\)HIPAD digitizing pad, Houston Instruments. Austin, Texas.
Figure 5-16 Equipment used to measure alterations within the periodontal ligament. NEC computer and digitizing pad.

Schematic illustrating a horizontal section depicting the interproximal periodontal ligament of the root filled tooth and the adjacent lateral incisor. Measurements of PDL thickness, cementum thickness, and vascularity were restricted to the interproximal regions of each tooth as depicted here.

Figure 5-17 Measurement of interproximal periodontal ligament. A specified area only was examined as depicted above (210μm X 140μm).
In order to determine if the root canal therapy had any bearing on the alterations to the periodontal ligament and its associated structures a second comparative parameter was introduced. This was the second lateral incisor. Thus for the seven animals comparisons were performed between the experimental teeth, the control teeth and the adjacent untreated second lateral incisor.

Statistical analysis of the tissue responses to the various amounts of orthodontic stimulation and retention was not performed due to the small number of animals used. However the correlation between the biological responses, assessed microscopically, and the observed clinical response will provide a more objective basis with which to apply the orthodontic root extrusion technique.
The following chapter has been divided into:

i. Clinical and radiographic findings of the orthodontic extrusion technique for the experimental and control teeth.

ii. Study model analysis of the amount of extrusion and relapse experienced by the experimental teeth.

iii. A descriptive analysis of the findings obtained from LM and SEM observation.

iv. A quantitative analysis of the alterations in cementum thickness, periodontal ligament width, and periodontal ligament vascularity for the experimental and control group obtained with selected SEM and LM sections. These findings have also been compared with the results obtained for the adjacent non root filled second lateral incisors.

6.1 CLINICAL FINDINGS

These findings include:

i. A description of the clinical appearance of the extruded teeth in comparison with the control teeth.

ii. A radiographic assessment of both the extruded and control teeth.

iii. Measurement of the amount of extrusion and relapse of the extruded teeth; and movement of the gingival margin of these teeth, obtained from study model analysis.

6.1.1 Clinical Appearance

The root filled teeth of the five experimental beagle dogs demonstrated the presence of chronic marginal gingivitis (figure 6-1, figure 6-2). Although a strict oral hygiene programme was developed and special dietary arrangements adhered to, the animals
still developed varying degrees of gingival inflammation. Of the two teeth in the control group, calculus deposition and chronic marginal gingivitis was also observed (figure 6-3).

Figure 6-1 Labial view of animal number three at the end of active extrusion. The orthodontic appliances(O) are in position and there is the presence of chronic marginal gingivitis with all of the anterior teeth (CMG).
Figure 6-2  Palatal view of animal number three. Chronic marginal gingivitis (CMG) is associated with the palatal gingiva of the anterior teeth. (ET) is the extruded tooth.

Figure 6-3  Labial view of control animal number six prior to sacrifice illustrating the presence of calculus (Cal) and chronic marginal gingivitis (CMG) associated with the anterior teeth. (ET) is the decoronated, non extruded control tooth.
Repeated appliance breakage was a feature associated with the experimental animals (figure 6-4). This was a difficult feature to control as the animals had a constant habit of chewing on their cage bars. Also, isolation of the animals was difficult as there was insufficient cage space. When allowed together during their exercise break, the animals often fought amongst themselves, thus increasing the incidence of broken orthodontic appliances.

![Image](image)

**Figure 6-4** Labial view of animal number two prior to appliance removal. There is chronic marginal gingivitis (CMG) present, and evidence of orthodontic appliance breakage (AB).

A feature encountered with two of the experimental animals (number 1 and 2) was the lack of space to extrude the experimental teeth. It was necessary in these two animals to decoronate two mandibular incisor teeth to provide sufficient room for extrusion of the experimental teeth (figure 6-5, figure 6-6). This problem was not encountered with the other animals.
Figure 6-5  Labial view of animal number one during appliance placement. The mandibular incisor (Inc) teeth were decoronated and endodontically treated.

Figure 6-6  Occlusion of experimental animal number one. Sufficient space for extrusion is present after decoronation of the mandibular teeth.
The total treatment period (including extrusion, retention, and waiting period prior to sacrifice) ranged from twenty seven to forty weeks (table 6-1). The animal with the shortest treatment period was beagle number five which underwent a periodontal surgery procedure in lieu of a retention period.

<table>
<thead>
<tr>
<th>Beagle</th>
<th>Initial</th>
<th>Treatment</th>
<th>Age</th>
<th>Sex</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>extrusive</td>
<td>period</td>
<td>(months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>force (g)</td>
<td>(weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>38</td>
<td>6</td>
<td>male</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>37</td>
<td>6</td>
<td>male</td>
<td>12</td>
</tr>
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<td>40</td>
<td>12</td>
<td>male</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>none</td>
<td>40</td>
<td>12</td>
<td>male</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 6-1 Table of initial extrusive force, age, sex, and weight of each animal at the beginning of the treatment period.

Of the five experimental teeth which underwent orthodontic extrusion, the animal with the greatest amount of tooth structure visible, and most defined margin tooth margin, was beagle number five.

Other than the presence of chronic marginal gingivitis, the clinical appearance of the teeth that underwent orthodontic extrusion was acceptable. No detrimental effects of either the root canal therapy performed or the root extrusion procedures instigated were observed clinically. No evidence of severe periodontal disease or excessive tooth mobility was clinically evident in the control teeth.
6.1.2 Radiographic Appearance

A successful root canal therapy was completed in all seven animals (figure 6-7, figure 6-8). The most characteristic radiographic finding of the teeth in the experimental group was the presence of a widened lamina dura at the completion of the four to six week active extrusion period (figure 6-9). This confirmed that movement of the experimental teeth had occurred after the active extrusion phase. The radiographic changes to the lamina dura and periapical region were not observed in the teeth of the control group (figure 6-8). It was observed that on completion of the retention period the radiographic changes present in the teeth of the experimental animals had decreased (figure 6-10). A feature observed in three of the five experimental animals (animals, 1, 4, and 5) was the development of a slightly widened lamina dura in the central incisor teeth adjacent to the experimental tooth (figure 6-9). This feature indicated that minor orthodontic movement of the adjacent teeth had occurred during the extrusion procedure.

![Radiographic Image](image)

**Figure 6-7** Preoperative radiograph of upper right first lateral incisor of animal one (Inc) prior to root canal therapy.
Figure 6-8  Periapical radiograph of the upper right first lateral incisor of control animal six illustrating the completion of a successful root filling (RF).
Figure 6-9  Periapical radiograph of the upper right first lateral incisor of animal one after the active extrusion phase. Widened lamina dura (LD) of the extruded tooth (ET) and the adjacent teeth present (ADJ).
Figure 6-10  Periapical radiograph of the upper right first lateral incisor of animal number one after the retention phase. The periapical radiolucency at the apex of the experimental tooth and adjacent teeth have decreased illustrating deposition of bone (Bone).

In summary, radiographs indicated successful completion of root canal therapy in the upper right lateral incisor of all seven animals. The decreased radiolucency at the apex of the extruded teeth indicated the presence of bone deposition.
6.1.3 Study Model Analysis

Study model examination confirmed that the experimental tooth in beagle number five which underwent a periodontal surgical procedure had the most complete tooth margins visible (figure 6-11). As illustrated in figure 6-11 the experimental teeth had quite swollen gingival papillae. Study model examination of the control teeth illustrated normal gingival contours (figure 6-12, figure 6-13). Study model examination of the control teeth illustrated normal alignment of the anterior teeth, and the decoronated root filled lateral incisor appeared intact and healthy (figure 6-12, figure 6-13).

It was difficult from study model examination to determine whether orthodontic movement of the incisor teeth adjacent to the experimental teeth had occurred during the extrusion procedure, as no implanted markers were positioned to accurately assess the changes (figure 6-14). Study model examination of the control teeth was unable to determine whether minor orthodontic movement of the adjacent teeth had occurred. As previously reported, periapical radiographs confirmed that minor movement of the adjacent incisor teeth had occurred during extrusion of the experimental teeth (figure 6-9).

![Figure 6-11](image)

**Figure 6-11** Labial view of study model of animal number five. Crown margins visible (CM). Brass points in position used for assessment of extrusion and relapse of the experimental tooth (BP).
Figure 6-12  Labial view of study model of control animal seven illustrating normal gingival contours. (C) is the decoronated control lateral incisor tooth.

Figure 6-13  Palatal view of control animal number seven illustrating normal gingival contours, and the decoronated lateral incisor tooth (C).
Figure 6-14 Palatal view of study model of animal number four. The central incisor teeth are observed to be palatally tilted. Brass points (BP).

6.1.4 Extrusion and Relapse of the Experimental Teeth

Study model examination illustrated that all five experimental teeth had signs of extrusive movement (table 6-2, graph 6-1). It was impossible to determine the true extrusive movement of these teeth without the presence of implanted radiographic markers. As mentioned in the previous chapter, a method utilising the study models was devised to measure the amount of extrusion of the experimental teeth relative to the adjacent central and second lateral incisor teeth.

<table>
<thead>
<tr>
<th>Beagle</th>
<th>Extrusion force (g)</th>
<th>Extrusion (mm)</th>
<th>Relapse (mm)</th>
<th>Final amount of extrusion (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>1.2</td>
<td>-1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>1.6</td>
<td>-0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>1.1</td>
<td>-0.1</td>
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</tr>
<tr>
<td>4</td>
<td>80</td>
<td>1.7</td>
<td>-1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>0.4</td>
<td>+0.34</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 6-2 Table of the amount of extrusion of the experimental teeth after the active extrusion period, relapse at completion of the waiting period, and final extrusion prior to sacrifice.
Graph 6-1  Graph of: a. The amount of tooth movement experienced by the experimental teeth after the active extrusion period (Active ext.), and b. The final degree of extrusion (Final ext.). Four of the animals experienced relapse. Animal five showed continued extrusive movement.

All five teeth of the experimental group experienced relative extrusive movement. The experimental teeth of beagle two and four appeared to have had the greatest amount of extrusive movement after the initial active tooth movement phase. These two animals had an initial extrusive force of 100 and 80 grams respectively. Animal five which had the greatest amount of initial extrusive force (120 grams), had the least amount of extrusive tooth movement. The +0.34mm value for animal five indicated that after the surgical procedure was performed on this tooth it continued to erupt. The experimental tooth in animal one, which had the least amount of initial extrusive force (30 grams) had the largest amount of relapse and smallest amount of final extrusive tooth movement.

In summary, relative orthodontic extrusion occurred in all five experimental teeth. Relapse was observed in four of these teeth. By contrast, the tooth which underwent a periodontal surgical procedure had continued tooth movement, and no relapse.
6.1.5 Movement of the Gingival Margin

Measurement of the study models was also used to determine the position of the gingival margin relative to the incisal edge of the extruded tooth (table 6-3).

The gingival margin was found to move with three of the experimental teeth. In experimental teeth one and four the gingival housing moved with the extruded tooth and maintained a constant relationship to the decoronated incisal edge of the tooth. With experimental tooth number two the gingival margin moved a portion of the overall extrusion distance the tooth moved. With experimental teeth three and five, the gingival margin did not move with the extruded teeth (graph 6-2). The gingival margin remained at a position very close to its original height in the case of animal three, but in the case of animal number five it actually moved further away from its original position by 0.4mm.

<table>
<thead>
<tr>
<th>Beagle</th>
<th>Distance of gingival margin to incisal edge of tooth prior to extrusion (mm)</th>
<th>Distance of gingival margin to incisal edge of tooth at completion (mm)</th>
<th>Distance gingival margin has moved relative to the incisal edge of the tooth (mm)</th>
<th>Final amount of extrusion</th>
<th>Overall distance gingival margin has moved</th>
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<td>0.7</td>
<td>-0.4</td>
</tr>
</tbody>
</table>

Table 6-3 Position of the gingival margin.
The gingival margin moved with the extruded tooth in three cases. The negative value for two of the animals indicated that the marginal gingiva has moved apically with respect to its original pre-extrusion level.

Graph 6-2  Graph of the movement of the gingival margin at the completion of treatment. The Y axis represents the original position of the gingival margin. Gridlines have been inserted to illustrate the movement of the gingival margin in animals 3 and 5 away from the incisal edge.

In summary, the gingival margin was found to move with three of the five experimental teeth (1, 2, 4). In the other two animals the gingival margin was found to move further away from their original position. In animal five the movement of the gingival margin away from its original position was the greatest at 0.4mm. It appeared that the animals with the heaviest forces of extrusion (animals 3 and 5) had the least movement of the gingival margin. The periodontal surgery procedure performed may have played a significant role in repositioning of the gingival margin in animal five.
6.2 DESCRIPTIVE ANALYSIS

6.2.1 Scanning Electron Microscope Findings

Three of the seven animals underwent scanning electron microscope analysis. These included two animals of the experimental group (animals 1 and 3) and one animal of the control group (animal 6).

6.2.1.1 Control Tooth

6.2.1.1.a Periodontal ligament and fibres

Longitudinal sections of the cervical region illustrated the presence of a normal junctional epithelium and gingival crevice. Supra-alveolar fibres of the periodontal ligament appeared to be orientated quite normally without any unusual stretching or adverse orientation (figure 6-15). No evidence of root or bone resorption was evident in the control tooth (figure 6-16, figure 6-17).

Longitudinal sections of the control tooth number six examined, demonstrated the presence of a narrowed periodontal ligament towards the middle third and apex of the periodontal ligament (figure 6-16). Figure 6-16 also illustrates that the blood vessels of the periodontal ligament of the control tooth in the region of the apex are difficult to discern. The interproximal periodontal ligament width appeared to reduce from 259μm at the cervical region to 81μm at the apical region of this tooth. These sections also demonstrated the presence of large marrow spaces, and occasional large vascular channels within the marrow space of the alveolar bone towards the apical region of the tooth. No evidence of alveolar bone resorption or tooth resorption was evident throughout the length of the tooth (figure 6-16, figure 6-17).

Horizontal sections of the control tooth number six illustrated the presence of a number of interesting characteristics. Descending the tooth from a coronal to a apical direction the following features of the interproximal PDL were observed:
i. The PDL appeared narrowed towards the apex of the tooth, consequently orientation of the periodontal ligament fibres could not be determined satisfactorily in horizontal sections (figure 6-18).

ii. The width of the periodontal ligament of the control tooth appeared to decrease in thickness towards the apex of the tooth as observed from horizontal sections (figure 6-18).

v. Evidence of secondary cementum deposition was noted towards the apex of the control tooth (figure 6-19).

6.2.1.1.b Vasculature

i. The vessels of the PDL in the control tooth appeared to be larger in diameter at the coronal third of the tooth but decreased towards the mid root region. The mean vessel diameter altered from 24μm at the cervical region to 12μm at the apex of the tooth (figure 6-20, figure 6-16).

ii. The vascular volume for the measured area decreased from 35911μm² at the cervical region to 11824μm² at the apical region (table 6-6).

iii. Figure 6-19 illustrates that a difference in blood vessel diameter is present between the experimental and adjacent non root filled lateral incisor.

6.2.1.2 Experimental Teeth

6.2.1.2.a Periodontal ligament and fibres

Periodontal ligament fibres of the experimental group indicated relatively normal orientation and direction. The fibres visible including those of the alveolar crest group, horizontal group, oblique group, and apical group were of a normal appearance. The cervical regions of the extruded teeth in the experimental animals did
not show significant evidence of increased marginal gingiva width or elongation of the periodontal ligament fibres (figure 6-21).

Longitudinal SEM views of the experimental teeth illustrated that the appearance of the junctional epithelium and the gingival crevice of the extruded teeth were very similar to the appearance of the control teeth. The supra-alveolar fibres of the gingival crevice and the dentogingival junction, appeared normal with no unusual orientation or elongation (figure 6-21, figure 6-22, figure 6-23). Figure 6-23 illustrates loss of gingival tissue interproximally, yet it does illustrate sufficiently the gingival crevice region.

6.2.1.2.b Cementum

The deposition of secondary cementum was a feature common to the two experimental teeth. There appeared to be greater cellular cementum apically for the sections of teeth examined (from 22μm to 64μm for beagle one and from 65μm to 77μm for beagle three). Cellular cementum deposition was present throughout the length of the extruded teeth (figure 6-24). Figure 6-24 also illustrates the difference in cementum width between the extruded tooth and the adjacent non extruded tooth to the left.

Experimental tooth three had evidence of interproximal cervical root resorption, (figure 6-25, figure 6-26). Root resorption was not observed in any other region of this tooth. Without histological views of this particular tooth it could not be determined whether this was active or inactive resorption. Cervical resorption was not observed in beagle number one (figure 6-22). No evidence of root resorption was observed in any other areas of the two experimental teeth.
Figure 6-15  Longitudinal SEM photomicrograph of the gingival crevice of animal six (control tooth). Supra-alveolar fibres (SA) of the periodontal ligament are orientated normally for both the control tooth (CT) and the adjacent second lateral incisor (ADJ). Gingival crevice (GC), Dentine (D), Mesial (M), Incisal (IN). Bar=1mm. Stereo pair, 6° tilt.
Figure 6-16  Montage of SEM photomicrographs of interproximal PDL of animal six (control tooth). Longitudinal view. The control tooth is on the right. There is no evidence of alveolar bone or cementum resorption. Alveolar bone (AB), Gingival fibres (G), Compressed PDL (P), Vascular channels (V), Mesial (M). Bar=1mm.
Figure 6-17  SEM photomicrographs of longitudinal section of control tooth illustrating the compressed periodontal ligament. Blood vessels (BV), Control tooth (CT), Alveolar bone (AB), Compressed PDL (P), Dentine (D), Periodontal ligament (PDL), Mesial (M), Incisal (IN). Bar=1mm. Stereo pair, 60° tilt.
Figure 6-18  SEM photomicrograph of horizontal section of interproximal region, mid root level, control tooth. There is a compressed appearance of the PDL and the lack of differentiation of the PDL fibres for the control tooth on the right. Alveolar bone (AB), Blood Vessels (BV), Control tooth (CT), Adjacent tooth (ADJ), Compressed PDL (P), Mesial (M), Buccal (B). Bar=100μm.
Figure 6-19  SEM photomicrograph of interproximal apical region of the control tooth. Horizontal section. There is present an altered size of blood vessels (BV) of the control tooth (CT) compared to the adjacent (ADJ) non root filled lateral incisor. Alveolar bone (AB), Cementum (CEM), Mesial (M), Buccal (B). Bar=100μm.
Figure 6-20  SEM photomicrograph of cervical interproximal region of control tooth, horizontal view. There are increased vascular channels of the control tooth on the right. Alveolar bone (AB), Blood vessels (BV), Control tooth (CT), Adjacent tooth (ADJ), Dentine (D), Mesial (M), Buccal (B). Bar=100μm.
Figure 6-21  SEM photomicrograph of gingival crevice region of beagle three, longitudinal view. The gingival crevice (GC) appears normal. Extruded tooth (ET), Adjacent tooth (ADJ), Mesial (M), incisal (I). Stereo pairs, 60° tilt. Bar=1mm.
Figure 6-22  Montage of SEM photomicrographs of animal one, longitudinal section. The extruded tooth is to the right (ET), a gradual increase in periodontal ligament vascularity (BV) can be observed as the apex of the tooth is approached. Alveolar bone (AB), Mesial (M), Incisal (IN).
Figure 6-23  SEM photomicrograph of interproximal region of animal one illustrating the gingival crevice (GC). Longitudinal section. Supra-alveolar fibres (SA), Experimental tooth (ET), Adjacent tooth (ADJ), Mesial (M), Incisal (I). Bar=1mm. Stereo pair, $60^\circ$ tilt.
Figure 6-24  SEM photomicrograph of interproximal region of animal three at the mid root level. Horizontal section. Note the presence of the larger blood vessels of the extruded tooth on the right (BV). There is also present uneven cementum (CEM) deposition in the interproximal region of the experimental tooth (EXP). Alveolar bone (AB), Dentine (D), Mesial (M), Buccal (B). Bar = 100μm.
Figure 6-25  SEM photomicrograph of interproximal cervical region of animal three. Horizontal view. Cervical resorption is present (RES). Experimental tooth (EXP), Gingival fibres (GF), Adjacent tooth (ADJ), Mesial (M), Buccal (B). Bar=100μm
Figure 6-26  SEM photomicrograph of interproximal region of animal three. High power horizontal view of the cervical resorption in animal 3. Note the extent of the resorption area (RES) and the presence of vascular channels (BV) within the PDL. Dentine (D), Mesial (M), Buccal (B). Bar=10μm.
6.2.1.2.c Vasculature

Horizontal sections illustrated that towards the middle and apical thirds of the experimental teeth large diameter blood vessels were present within the periodontal ligament. The larger diameter blood vessels were usually situated within the outer third of the periodontal ligament (figure 6-24). In animal three the blood vessels were found to have a mean diameter of 15μm at the cervical region, 9μm in the mid root region, and 12μm at the apical region. In animal three the smallest diameter blood vessel was 5μm while the widest blood vessel was 16μm. In animal one the blood vessels were found to have a mean diameter of 10μm at the cervical region, 12μm in the mid root region and 18μm at the apical region. In animal one the blood vessels ranged in diameter from 11.2μm to 18μm.

The alteration in the vascular morphology of the extruded tooth was quite obvious in animal number one. Compared to the second lateral incisor (non root filled) the blood vessels of the extruded tooth were larger (figure 6-27). The increased vascular area, and size of the blood vessels was quite noticeable, and as the apex of the tooth was approached these vascular regions became increasing larger. The mean interproximal vascular area for beagle number one increased from 22298μm² at the cervical region of the tooth to 65366μm² at the apex of the tooth (table 6-6). In contrast the mean interproximal vascular area of the non root filled lateral incisor only increased from 5570μm² to 19167μm² (figure 6-28). Figure 6-24 also illustrates that the vessels within the periodontal ligament of the extruded tooth in beagle three were larger than the adjacent non root filled tooth. The vascular area in beagle three decreased from 32020μm² at the cervical region to 6444μm² at the apical region. Further discussion of vasculature will be presented in section 6.3.1.

In general alterations to the vasculature of both the experimental and control teeth had occurred. A description of the control teeth has already been given. The changes appear to include alterations to vascular size and blood vessel number.
Figure 6-27  SEM photomicrograph of interproximal region of beagle one, mid root level. Horizontal view. Note the alteration in vascularity (BV) of the extruded tooth (EXT) on the right. Alveolar bone (AB), Dentine (D), Mesial (M), Buccal (B). Bar = 100µm. Stereo pair, tilt is 6°.
Figure 6-28  SEM photomicrograph of interproximal apical area of beagle one. Horizontal section. Blood Vessels (BV), Alveolar bone (AB), Periodontal ligament (PDL), Mesial (M), Buccal (B). Bar = 100μm. Stereo pair, 60° tilt.
6.2.1.3 Features of the Extracted Tooth Half

SEM examination of the extracted half of the experimental and control teeth did not show any unusual features. Specimen preparation was poor due to the difficulty of separating an intact half of the tooth in one piece. However, these teeth appeared intact and sound. No evidence of extensive root resorption or tooth destruction was observed in the experimental tooth number one (figure 6-29). Due to poor specimen quality photographs of animal three and six were unable to be provided.

6.2.1.4 Summary

SEM analysis of the extruded and control teeth has provided a number of interesting features. The control tooth illustrated a decreased periodontal ligament width towards the middle third and apical third of the tooth. Alterations in vascular morphology were observed, including decreased blood vessel size and diameter towards the middle and apical third of the control tooth. The two experimental teeth and the control tooth illustrated the presence of increased cellular cementum deposition towards the apex. The most interesting feature observed was the increased interproximal vascularity of the extruded teeth. Cervical resorption was noted interproximally in one of the experimental teeth. Periodontal ligament fibre orientation of the experimental and control teeth appeared normal. There was also present a narrowed periodontal ligament of the control tooth which provided difficulty in examining the vasculature. It was difficult to determine whether the narrowed appearance of the periodontal ligament in the control tooth was due to a processing fault or due to the root canal therapy.
Figure 6-29  SEM photomicrograph of extracted half of tooth from animal number one. Scratches and markings from the processing procedure are present throughout the specimen (SCT). There is no indication of long term damage caused by the extrusion technique. Bar=100μm.
6.2.2 Light Microscope Findings

Four of the seven animals treated underwent histological examination. Three of these animals were part of the experimental group (two, four, and five) and the other animal was part of the control group (seven). Histological examination confirmed many of the findings determined by SEM examination.

6.2.2.1 Control Tooth

One of the main problems encountered with the control tooth was the difficulty in staining with haematoxylin and eosin. This problem proved to be present with all of the sections prepared for histological examination. Consequently the information obtained was not as great as expected.

6.2.2.1.a Periodontal ligament

Horizontal sections illustrated Sharpey's fibres to be seen passing from the alveolar bone through the periodontal ligament and extending into the cementum of the control tooth. Histological examination of the control tooth observed normal alignment of the periodontal ligament fibres. The fibres appeared to be aligned without unusual variation or elongation.

6.2.2.1.b Cementum

Cementum deposition was observed towards the middle and apical thirds of the control tooth (figure 6-30). Interproximal cementum thickness increased from a mean width of 63μm at the cervical region to 154μm at the apical region of the control tooth (table 6-7). Much more cementum deposition was evident in this control tooth than the control tooth one examined with scanning electron microscopy.
6.2.2.1.c Alveolar bone

No evidence of alveolar bone or root resorption could be found throughout the length of the control tooth. This was in agreement with the findings observed with SEM analysis of the control tooth number six. Large marrow spaces were also evident in the marrow spaces of control tooth number seven (figure 6-30). This contrasted with control tooth number seven examined via scanning electron microscopy.

6.2.2.1.d Vasculature

The main blood vessels of the control tooth were venular in origin. Very little difference in blood vessels diameter could be found between the control tooth and the adjacent non root filled lateral incisor (figure 6-30, graph 6-7).

6.2.2.2 Experimental Teeth

6.2.2.2.a Alveolar bone

Significant alveolar bone deposition was observed in the interproximal region of the extruded tooth of beagle five only (figure 6-31). No evidence of ankylosis was observed clinically, nor histologically (figure 6-31). Histological views did not illustrate the presence of osteoclastic cells (figure 6-38). No evidence of alveolar or apical bone deposition could be located in the other experimental animals or the control animal. Wide variation in the size of the interproximal marrow spaces were observed between the experimental and control teeth (figure 6-30 and figure 6-32). Interproximal bone deposition was unable to be assessed adequately as the horizontal sections proved inadequate to examine adequately.

6.2.2.2.b Cellular Content

Horizontal and longitudinal sections illustrated that the majority of the cells in the periodontal ligament of the experimental and control teeth were fibroblasts. The fibroblasts present were of the flattened and plump type signifying both active and
inactive forms (figure 6-33). No evidence of inflammatory cells could be found in either the control or the experimental group. No difference in cellular component between the experimental and the control teeth was observed. High power histological views did not show the presence of osteoclastic or cementoclastic cells within the resorptive sites. The predominant cell within the periodontal ligament of the experimental and control teeth was the fibroblast.

6.2.2.2.c Vasculature

The blood vessels of the periodontal ligament of both the control and experimental teeth were mainly of the venular form (figure 6-33, figure 6-34). Only occasional evidence of arterial blood vessels could be found in the histological sections examined (figure 6-34). They were quite difficult to locate and there was little evidence of arterial blood vessels in any of the root filled teeth, control or experimental (figure 6-350. As with the teeth observed under the scanning electron microscope, larger vessels were observed towards the middle and apical thirds of the periodontal ligament. The larger vessels of the experimental teeth were found to be situated towards the outer third of the periodontal ligament, closer to alveolar bone than cementum. Examination of the cell wall thickness and endothelial cell structure confirmed that these larger vessels appeared to be venular. Neurovascular bundles incorporating blood vessels and a neural element were occasionally located in the periodontal ligament of the non root filled second lateral incisor (figure 6-34). These neurovascular bundles were difficult to locate in the experimental and the control teeth and appeared to be very few in number. A feature of the extruded teeth was the presence of larger blood vessels in the outer third of the periodontal ligament in some of the sections examined (figure 6-35). An unusual feature observed with some of the histological sections examined was the possible communication of the periodontal ligament vasculature across the interseptal alveolar bone to the adjacent periodontal ligament. Histological sections did not illustrate a clear vascular communication,
however it was an interesting observation that requires further investigation (figure 6-36).

6.2.2.2.d Cementum

Cellular cementum deposition was also noticed towards the middle and apical third of the experimental teeth observed with histological analysis (figure 6-37). This confirmed the findings observed with SEM analysis. Cellular cementum deposition was a common finding of the extruded control teeth, and it was markedly thicker towards the apical regions of the teeth (table 6-7).

Interproximal cervical resorption was observed in two of the three experimental teeth. Cervical resorption was found to be arrested with no histological evidence of cementoclasts (figure 6-38, figure 6-39). The animal with the greatest amount of cervical root resorption was animal number five (figure 6-40). Longitudinal sections confirmed the presence of cervical resorption in animal five (figure 6-41). Experimental tooth number four also had interproximal cervical resorption present, though not to the same extent as tooth number five. No evidence of root resorption could be found in any other regions of the experimental or control teeth.
**Figure 6-30** LM photomicrograph of control tooth, horizontal section, mid root. Root filled tooth is to the right, cementum (CEM) deposition can be observed. Periodontal ligament (PDL), Blood vessels (BV), Mesial (M), Buccal (B). Haematoxylin and eosin. Magnification X50.
Figure 6-31  LM photomicrograph of animal number five, interproximal cervical region. Horizontal section. Alveolar bone deposition (AB) extending into the resorption area of beagle five. Extruded tooth (EXT), Adjacent tooth (ADJ), Blood vessels (BV), mesial (M), Buccal (B). Haematoxylin and eosin. Magnification X100.

Figure 6-32  LM of photomicrograph of interproximal region between the upper right first and second lateral incisor of animal two, mid root level. Horizontal section illustrating increased vascular supply (BS) to the extruded tooth (ET) on the right. Alveolar bone (AB), Dentine (D), Mesial (M), Buccal (B). Haematoxylin and eosin. Magnification X250.
Figure 6-33  LM photomicrograph of animal two at the interproximal mid root level. Horizontal section. Fibroblasts present within the periodontal ligament. Dentine (D), Plump fibroblasts (PF), Flattened fibroblasts (FF), Periodontal ligament (PDL), Sharpey's fibres (SF), Mesial (M), Buccal (B). Haematoxylin and eosin. Magnification X500.

Figure 6-34  LM photomicrograph of the periodontal ligament of the non root filled second lateral incisor of animal two illustrating a vascular bundle incorporating blood vessels (BV) and neural supply (NS). Dentine (D), Arteriole (A), Mesial (M), Buccal (B). Haematoxylin and eosin. Magnification X250.
Figure 6-35  LM photomicrograph illustrating the periodontal ligament of the extruded tooth in animal five. Horizontal section illustrating increased vessel size of the extruded tooth on the right. Large blood vessels (BV) are situated towards the outer third of the periodontal ligament (PDL). Arteriole (A), Sharpey's fibres (SF), Mesial (M), Buccal (B). Tri-chrome stain. Magnification X500.
Figure 6-36  LM photomicrograph of animal number 4 mid root level, horizontal view. There appears to be a vascular channel (VC) communicating between the experimental tooth (ET) to the right, and the adjacent non root filled incisor to the left. Mesial (M), Distal (D), Buccal (B). Magnification X500.

Figure 6-37  LM photomicrograph of animal number four mid root level. Horizontal view. Cementum deposition (Cem) of the extruded tooth (ET) on the right. Sharpey's fibres visible (SF). Tri-chrome stain. Magnification X250.
Figure 6-38  LM photomicrograph of interproximal cervical region of animal four. Horizontal view. Alveolar bone (AB), Adjacent tooth (ADJ), Dentine (D), Experimental Tooth (EXP), Resorption (RES), Mesial (M), Buccal (B). Haematoxylin and eosin. Magnification X50.
Figure 6-39  LM photomicrograph of distal surface of the upper right first lateral incisor of animal five. Horizontal section, cervical region. Cervical bone deposition (AB) adjacent to an area of root resorption (RES) in tooth number five. Blood vessels (BV), Dentine (D), Mesial (M), Buccal (B). Haematoxylin and eosin. Magnification X250
Figure 6-40  LM photomicrograph of distal cervical region of upper right first lateral incisor of beagle five. Horizontal section. High power view of cervical resorption present in animal number five. Alveolar bone (AB), Blood vessel (BV), Dentine (D), Resorption (RES). Haematoxylin and eosin. Magnification X500.
Figure 6-41  LM photomicrograph of distal surface of the upper right first lateral incisor of animal five, longitudinal section. Cervical resorption (RES), Gingival crevice (GC), Dentine (D), Mesial (M), Incisal (IN). Haematoxylin and eosin. Magnification X50.
6.2.2.3 Tables of Results, Calculated from SEM & LM Specimens

It must be remembered that the results obtained in the following tables were calculated from a region measuring only 210 X 140μm². The results are only intended to provide an indication of the alterations to the periodontal ligament, and are not to be taken as indicative of the entire interproximal periodontal ligament.

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Table 6-4 Table of blood vessel numbers for the cervical, mid root and apical regions of the experimental and control teeth.

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Table 6-5 Table of mean blood vessel diameter for the cervical, mid root and apical regions of the experimental and control teeth.
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Table 6-6: Table of the total interproximal vascular area for the cervical, mid root and apical regions of the experimental and control teeth.

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<th>BEAGLE</th>
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Table 6-7: Table of cementum thickness for the cervical, mid root, and apical regions of the control and experimental teeth.
6.2.2.4 Summary

Histological analysis of the experimental and control teeth revealed the presence of cervical resorption in three of the experimental teeth. Cementum deposition was noticed in both the experimental and control teeth. No evidence of inflammatory cells could be located in either the experimental or control teeth. Fibroblasts were the predominant cell in the experimental teeth and were present in both the active and inactive form. These vessels were mainly of the venular form. Arterial blood vessels were uncommon in horizontal and longitudinal sections.

6.3 Quantitative Analysis

The following analysis was based on three horizontal sections for the experimental and control teeth to represent the cervical, mid root and apical regions of that tooth. The results have been presented in the form of graphs to illustrate if a trend existed for alterations occurring within the various regions of each tooth. A comparative analysis has been presented to compare the different regions between the experimental and the control teeth. A comparison has also been made between the root filled teeth and the non root filled second lateral incisor. This was performed to determine if any difference existed between the root filled and non root filled teeth. The results should not be interpreted as representing the tooth in its entirety. These results are intended to confirm many of the observations already observed. Due to the small sample size a statistical analysis was not performed. An examination of the changes in periodontal ligament width, cementum thickness, blood vessel number and size, and vascular area will be presented. The data used to obtain many of the following results is located in Appendix 4.

Unless otherwise stated the measurements for PDL width, cementum thickness and vessel diameter will be in μm and vascular area will be in μm².
6.3.1  Vascular Changes

6.3.1.1  Blood Vessel Numbers

There was no consistent trend in the number of blood vessels between the experimental and the control teeth (graph 6-3).

![Blood vessel numbers graph](image)

**Graph 6-3**  Graph illustrating the difference in the number of blood vessels between the cervical, mid root and apical regions of the experimental and control teeth (210μm×140μm).

Experimental tooth one had an increased number of blood vessels cervically. Experimental teeth three, and four had an extremely large number of blood vessels in the cervical and mid root region. It appeared that the number of blood vessels in the apical region of these teeth was relatively less than other regions of the teeth. Control teeth six and seven, and experimental tooth two and five had a fairly consistent number of blood vessels throughout the various regions measured.

When the number of blood vessels in the different regions of the adjacent non root filled second lateral incisor were examined there was a pattern that appeared similar to the results obtained for the root filled teeth (graph 6-4).
Graph 6-4  Graph illustrating the number of blood vessels in the cervical, mid root and apical regions for the adjacent non root filled teeth second lateral incisor (pure numbers of blood vessels).

The distribution of blood vessels and the number of blood vessels appeared similar for both the experimental teeth and the adjacent non root filled lateral incisor teeth. In animals one and two the pattern of distribution appeared similar. In animals three and four there was a greater number of blood vessels for both the experimental teeth and the adjacent non root filled teeth compared with the other experimental teeth. In teeth five, six, and seven the numbers of blood vessels in the root filled teeth and the adjacent teeth appeared similar, with the pattern of distribution being also similar.

When the total number of blood vessels from the three different regions were calculated, the root filled teeth were compared with the non root filled second lateral incisors. It was observed that the number of blood vessels for the non root filled second lateral incisors appeared greater than for the extruded tooth in animals two, four, five, and six, although the difference appeared to be minor (graph 6-5).
Sum of the blood vessels for the root filled teeth and their adjacent non root filled teeth

Graph 6-5  Graph illustrating the total number of blood vessels from the three regions previously described for the root filled teeth and the adjacent non root filled second lateral incisor.

6.3.1.2 Blood Vessel Diameter

In comparing blood vessel diameter there appeared to be no relationship present between the experimental and the control teeth. In general the blood vessels diameters for the teeth examined via scanning electron microscopy appeared less than those examined histologically. This feature may have been a consequence of the different processing procedures. In animal one the blood vessel diameter increased apically. In animals two, three, and four, the mid root vessel diameter was less than the adjacent regions. This was the converse in animal five where the mid root vessel diameter was greater than the adjacent regions. In animal six the apical blood vessel diameter decreased, and in animal seven the vessels diameter appeared reasonably constant. Generally, blood vessel diameter appeared to vary greatly between different regions of each tooth and between individual animals (graph 6-6).
Graph 6-6  Graph illustrating the mean blood vessel diameters from the selected interproximal regions for the cervical, mid root and apical regions of the control and experimental teeth.

When the mean blood vessel diameters for the root filled teeth were compared with their adjacent non root filled second lateral incisors there was a slight trend for an increase in mean blood vessel diameter of the root filled teeth for four of the five experimental animals (graph 6-7). In animal three the blood vessel diameter of the experimental tooth was less than the adjacent non root filled tooth. In control teeth six and seven, one animal was found to have a greater blood vessel diameter for the root filled tooth, while the opposite was found in animal seven.
Graph 6-7 Graph illustrating the mean blood vessel diameter between the root filled experimental and control teeth compared with the adjacent non root filled teeth.

In summary there appeared to be a trend for increasing blood vessel diameter towards the apex in three of the experimental teeth. When comparing the mean blood vessel diameter of the root filled teeth with their adjacent non root filled second lateral incisors there was a general trend for the mean vessel diameter of the root filled teeth to be slightly greater than the non root filled second lateral incisors in five of the animals.

6.3.1.3 Vascular Area

There appeared to be a decrease in vascular area for the apical regions of experimental teeth three, four, and five. In animals one and two there was a gradual increase in vascular area. The two control teeth also illustrated a decrease in apical vascular area (Graph 6-8).
Graph 6-8  Graph illustrating the variation in vascular area between the cervical, mid root and apical regions of the experimental and control teeth. Vascular area measured in μm².

In beagle one and two there was an increase in vascular area from the cervical region to the apical region of the teeth. This was in marked contrast to the other teeth. The vascular area for control teeth six and seven appeared to be less than the experimental teeth with the exception of animal three. In animal four there was significantly greater vascular area measured for the cervical and mid root regions.

In examining the vascular area of the adjacent non root filled teeth there was also great variation present. The pattern of vascular area appeared to have a similar appearance with those of the root filled teeth with the exception of teeth two and three. The experimental tooth two had an increase in vascular area apically, whereas the adjacent second lateral incisor tooth had a decrease in vascular area apically. In animal three the converse was observed (graph 6-9).
Graph 6-9  Graph illustrating the vascular area between the selected interproximal cervical, mid root and apical regions of the adjacent non root filled second lateral incisor. Vascular area measured in \( \mu m^2 \).

When the total vascular area of the root filled teeth was compared with the adjacent non root filled second lateral incisor there appeared to be an increased vascular area for the root filled teeth. This was noticeable in experimental teeth one, two, and four (graph 6-10). The two control teeth maintained a constant vascular area when compared with their adjacent non root filled second lateral incisors.

Graph 6-10  Graph illustrating the sum of vascular areas between the root filled and adjacent non root filled teeth. Vascular area measured in \( \mu m^2 \).
In summary, the vascular alterations indicated that three of the root filled extruded teeth (one, two, and four) had a greater vascular area than the two root filled control teeth (six, and seven), and four of the experimental teeth (one, two, four, and five) had a greater vascular area than the non root filled adjacent second lateral incisor. Variation was observed to be present between the different regions of each experimental tooth. No consistent trend between the extruded teeth, the control teeth, and the adjacent second lateral incisor was observed, although this may have proved to be more obvious in a greater sample size.

6.3.2 Periodontal Ligament Width

The periodontal ligament width was quite consistent in specimens one, two, four, five and seven. In tooth three the apical PDL width was wider than the cervical region, and in tooth six the cervical region was wider than the apical region. The compressed appearance observed in the control specimen number six may have contributed to the wide variation in width between the PDL width cervically and apically. No obvious trend was observed between the control group and the experimental group (graph 6-11).

![Graph 6-11](image)

**Graph 6-11** Graph illustrating the variation of PDL thickness between selected interproximal regions of the cervical, mid root and apical regions of experimental and control teeth.
A comparison of the periodontal ligament width between the experimental and control teeth, with the adjacent second lateral incisor teeth was performed to determine if there was any variation between the root filled and non root filled teeth (graph 6-12).

Graph 6-12 Graph illustrating the difference in PDL width from the selected interproximal regions between the root filled and non root filled teeth.

There was a trend for the width of the periodontal ligament for both the root filled tooth (RCT tooth) and the adjacent non root filled (Adj. tooth) to be similar.

In summary, it appeared that the periodontal ligament thickness of the experimental teeth was greater than that of the control teeth. When comparing the adjacent non root filled second lateral incisor with the experimental teeth, it could be seen that the periodontal ligament width of those teeth appeared similar to the periodontal ligament width of the experimental teeth.

6.3.3 Cementum
Wide variation in cementum width was found between individual regions of the experimental teeth and individual teeth, although there was a trend for increased thickness of cellular cementum deposition towards the apex of the extruded teeth and
control teeth (graph 6-13). In animals one, two, five, six, and seven there was increasing cementum width apically. In animals three, and four no uniform trend was obvious.

![Variation in cementum thickness](image)

**Graph 6-13** Column graph illustrating the increasing thickness of the cementum towards the apical region of the experimental and control teeth.

Examination of the alterations in cementum thickness between the adjacent non root filled second lateral incisor also indicated the presence of thickened cementum towards the apex of the teeth, although the cementum thickness was considerably less than the root filled teeth (graph 6-14).
Variation in cementum thickness between regions of the non root filled teeth

Graph 6-14 Graph illustrating the alterations in cementum thickness in selected interproximal regions of the adjacent non root filled second lateral incisor.

In animals one, two, three, and five, there was a steady increase of cementum thickness apically. In animal six the cementum thickness appeared considerably less than the other teeth, this may have been due to the compression of the periodontal ligament, and may indicate a possible specimen processing problem. In control animal seven the thickness of the cementum for the non root filled lateral incisor was comparable to the root filled tooth, and also much greater than the other non root filled teeth.

A comparison of the mean cementum width between the experimental and control teeth, with the mean cementum width of their adjacent non root filled second lateral incisors was performed (graph 6-15).
Comparison of the cementum widths between the experimental and control groups, and their adjacent counterparts

Graph 6-15  Comparison of cementum thickness from the selected interproximal regions between the experimental and control group teeth, and their adjacent non root filled second lateral incisors.

The most notable feature in cementum thickness was the increased width of the cementum towards the apex of the experimental teeth. This confirmed many of the observations from the LM and SEM examination. Apart from one control tooth (number 7), the cementum thickness of the adjacent non root filled second lateral incisor teeth, was notably less than those of the root filled extruded teeth.

In summary, the quantitative analysis has indicated that the orthodontic extrusion technique appears to have lead to long term alterations within the interproximal periodontal ligament. Alterations to periodontal ligament vascularity have occurred without any one feature being most prominent. There appears to have been minor alteration in periodontal ligament width between the experimental and control teeth. It must also be remembered that the PDL of the control tooth examined by SEM illustrated a compressed PDL. This feature may have thus contributed to the different PDL widths observed. Finally there appeared to be a gradual increase in cementum thickness from the cervical region to the apical region of all seven root filled teeth. This feature contrasted markedly with the non root filled second lateral incisor teeth.
7 DISCUSSION

7.1 TECHNICAL CONSIDERATIONS

7.1.1 Decoronation

Decoronation with a high speed bur in the present project was easy and effective, though it did not realistically represent the clinical setting where a crown-root fracture of a tooth occurs after a traumatic incident. This feature may have had some bearing on the results obtained because a crown-root fractured tooth usually suffers injuries to the dental pulp, and the periodontium. As the teeth in our experimental situation did not receive a traumatic injury, the response of the periodontium may be different in a traumatic clinical setting. However, decoronation with a bur did allow us to determine the biological responses subsequent to root extrusion. We were able to determine a "normal response" to our understanding of the extrusion procedure without the associated trauma to the soft tissues. This factor requires due consideration for further research, where the crown-root fracture of a tooth may be initiated with a blow from a blunt instrument.

7.1.2 Perfusion and Fixation

One of the technical problems encountered concerned the fixation and perfusion process. The seven beagle dogs demonstrated clinical signs of suitable fixation, including blanching of the jaws, and rigidity of tissue tone. When the specimens were processed for histological examination, many of the blood vessels were found to have clumping of red blood cells. The blood vessels were noticed on some histological specimens to have disrupted cell walls, thus making the process of vessel identification extremely difficult.

One of the aims of the present project was to provide the author with initial experience in the histological processing of slides. As evidenced in figure 6-30 there were
problems associated with the staining of histological specimens. With the aid of
technical staff, and practice on extruded teeth a successful laboratory technique was
achieved. Also, when the appropriate regions of the maxilla were removed, blood
mixed with fixative was noticed to be oozing from the cut tissues. The majority of the
histological sections examined were free of red blood cells, consequently the process
could be classified as adequate. In summary the completion of this project provided
the author with valuable information concerning the production of histological slides
and the processing of specimens for laboratory investigation.

7.1.3 Specimen Preparation
Infiltration and histological processing with LR White\(^1\) proved to be a difficult
procedure. LR White was used in this project because the manufacturer considered
that it provided superior tissue embedding properties and staining characteristics
compared with paraffin. LR White proved to be extremely difficult to cut successfully
with the microtome into thin sections, and it was after much trial and error, involving
repeated sectioning of unwanted tissue regions to determine the most appropriate
width of sectioning, that a successful technique was developed. The manufacturer of
LR White advised the use of prolonged staining periods when preparing the
histological specimens\(^2\). The present author did not consider the manufacturers
recommended method of staining with haematoxylin and eosin to be adequate, and
consequently devised an alternative method of histological staining as detailed in
chapter five. The main differences between the presently developed technique and the
manufacturers technique involved the use of Mayers Haematoxylin instead of Harris
Haematoxylin, and shortened staining times from 60 minutes to 30 minutes. Once the
technique was mastered the colour and staining of the resin sections provided
excellent results. It appeared that the use of acrylic resins for histological processing
of dental structures has considerable merit.

\(^1\)London Resin Company, Surrey.
\(^2\)Using LR White for hard tissue. London Resin Company, Surrey.
Problems were also encountered with the processing of specimens for SEM analysis. The endodontically treated teeth appeared extremely brittle and friable. This factor was clearly illustrated in the SEM photographs, where longitudinal montages illustrated cracking and friability of the dental structures (figure 6-16). This was particularly prevalent in the apical regions, and hampered adequate examination of these areas. The use of stereo pair photography for certain regions such as the gingival crevice, and the periodontal ligament, was advantageous as it has been considered that stereo pair photography represents an increase in the availability of information from micrographs (Wergin and Pawley, 1980). Stereo pair photography was utilised for all sections examined by scanning electron microscopy in the present project. Only the sections of the highest quality have been reproduced.

7.1.4 Radiographic Measurement

When the project was initiated, a standardised radiographic technique was not performed. A Formasil\(^1\) key was constructed to allow repositioning of the radiographic film for each successive radiograph, although a standardised horizontal and vertical plane were not employed. The absence of implanted markers increased the problems of measuring the amount of extrusion experienced by the experimental teeth. The method used to overcome this will be discussed in section 7.6.

7.1.5 Animal Model

An experimental animal in any research project should be easy to handle and the results obtained should also be able to be extrapolated to the human situation (Barker, 1970). There have been reported difficulties in the use of animals for endodontic and orthodontic research, as it is not always possible to produce experimentally the various pathological changes which affect humans (Barker, 1970). Handling of animals has always been a major concern when using animals for research. The

\(^{1}\)Silicon based impression material. Kulzer, Germany.
difficulties of handling are minimised when small animals such as rats and guinea pigs are used. However with small animals there is not only the difficulty of access to the pulp chambers but also the altered healing patterns that differ from those of humans. The marked healing capacity exhibited by the traumatised rodent pulp is not found in human teeth (Barker, 1970).

Weir (1991) reported that, one of the difficulties they experienced on a TEM study (Weir and Sims, 1991) concerning the extrusion of marmoset teeth, was the completion of a successful root canal therapy. Weir (1991) expressed that one of his concerns was that the apical anatomy of marmoset incisor teeth prevented adequate pulp extirpation and instrumentation towards the last 0.5mm of the tooth. By contrast Barker (1970) concluded that "endodontic experimentation which produces healing in the dog can be taken to suggest acceptable therapy in man". The morphology of the beagle dog incisor was favourable for a project utilising root filled teeth. The incisor teeth in the beagle dogs were approximately fourteen millimetres in length. The length and shape of the pulp chamber were not quite the same as a human tooth, nevertheless the morphology did not preclude the successful completion of a root canal therapy in the present project. The use of gutta percha as a root canal sealant was a complete success in the present study as evidenced radiographically. The successful completion of root canal therapy in the beagle dogs confirmed the statement of Barker (1970) that "there is no evidence that the periapical tissues in the dog or human react in a significantly different manner to endodontic procedures".

7.2 THE EXTRUSION DEVICE

Problems were encountered maintaining the extrusion device in position. With regular maintenance and care the beagle dogs still managed to damage their orthodontic appliances. Other researchers have encountered problems with maintaining orthodontic appliances in position in research animals (Botting and Storey, 1973). They did not mention how the problem was overcome.
Previous research has not utilised conventional orthodontic appliances to extrude decoronated root filled teeth. Batenhorst et al. (1974) investigated the changes in hard and soft tissues relative to facial movement of incisors in monkeys. By coincidence they also achieved extrusive movement of these teeth. Batenhorst et al. (1974) utilised conventional bonded orthodontic appliances to extrude teeth, however their study performed was on vital teeth. Simon et al. (1980) utilised cemented cap splints to extrude premolar teeth in dogs, they did not report any associated problems with their technique. Crowe (1989) utilised magnets to extruded incisor teeth in cotton eared marmosets. Weir and Sims (1991) utilised samarium-cobalt magnets cemented into decoronated root filled marmoset teeth. Individually cast cobalt chrome devices were bonded to the palatal surfaces of the other teeth and a magnet was placed into this retainer. Weir (1991) reported regular breakage of the extrusion appliance, which he attributed to unusual masticatory habits including the chewing of their cage bars. Short term extrusion studies carried out on rats by Lew et al. (1988), and Cooper and Sims (1989), were performed with specifically modified extrusion devices consisting of modified tweezer beaks with a quick release system. These short term studies performed lasted for only thirty minutes, and as such no evidence of appliance breakage was reported.

Another problem with the extrusion device was that it caused laceration and tearing of the adjacent oral soft tissues. Trauma to the soft tissues may have contributed to the unusual chewing habits of the beagles such as chewing on the cage bars in an attempt by the animals to dislodge the orthodontic appliances. The dislodgement of the orthodontic appliances ultimately lead to the discontinuation of three of the original ten animals.

The advantage of utilising orthodontic brackets to extrude the teeth in this project was that it simulated many of the clinical scenarios that would be experienced by human patients having to undergo extrusion of a crown-root fractured tooth. This is
particularly so in relation to the amount of force utilised for extrusion, as well as the
direction of the force. Also the appliances were applied to teeth of similar size to
human incisors.

From the present study one of the technical problems that will require further
improvement involves the extrusion device. The use of cemented cap splints may be
more beneficial for long term extrusion procedures. It may be that, regardless of the
appliance used for extrusion, the masticatory habits of the research animals, including
chewing on their cage bars, and fighting with each other may ultimately cause
dislodgement of the appliance, no matter how well it was retained in position.

7.3 EXTRUSION FORCE

One factor which could not be controlled adequately was the long term extrusive force
placed on the experimental teeth. The extrusive force was easy to apply initially
although as the animals damaged their orthodontic appliances during the extrusion and
retention periods, it was difficult to control the actual force placed on the experimental
teeth. Consequently, it was difficult to determine whether there was any clinical
difference between light and heavy forces of extrusion. This was one detriment of the
 technique utilising bonded orthodontic brackets. Measurement of the amount of
extrusion experienced by the five experimental teeth indicated little difference between
the teeth after the four to six week extrusion period.

The amount of force used to extrude decoronated root filled teeth has been given little
attention in the dental literature. Cooke and Scheer (1980) advocated that 70 to 150
grams of force be used to extrude a non vital tooth. Proffit (1986) suggested that 50
to 100 grams of force be applied to extrude a vital tooth. Other authors have not
recommended a particular force to extrude non vital teeth (Heithersay, 1973;
Biggerstaff et al., 1986). Most authors determine that the amount of extrusion
required is relative to the depth of the palatal fracture, in other words time and distance
related rather than force related (Heithersay, 1973; Simon, 1984). Heithersay (1973) considered that orthodontic extrusion could be complete within four weeks. Simon et al. (1978) considered that extrusion could be complete within one to three weeks. Simon et al. (1980) observed that extrusion occurred within a week of placing orthodontic tension on premolar dog teeth, although the amount of extrusion was not measured. The extrusion protocol for the experimental animals in this present study was only four to six weeks.

It was apparent from the present study that extrusion of root filled teeth in beagle dogs can be achieved within four weeks. The exact duration for extrusion required in a crown-root fractured situation must depend on the depth of the palatal fracture, and on the amount of tooth movement that is necessary. From the present study it appeared that regardless of the initial extrusive force applied to the five experimental animals, they had a similar degree of tooth movement upon completion of the extrusion period. It can be inferred that it is the period during which an orthodontic force is applied which is more significant than the actual force applied in extruding teeth. Moving teeth a greater distance requires greater duration of force not greater amount of force.

As the orthodontic appliances were positioned from second lateral incisor to second lateral incisor, the question of reciprocal intrusive movement of the adjacent teeth was considered. Study models observed possible movement of the adjacent central incisor teeth. Stern and Becker (1980) felt that very little significant intrusive movement of the adjacent teeth occurs during orthodontic root extrusion as the reciprocal intrusive forces are dissipated amongst all of the anchor teeth. This appeared to be the case in the present project, where movement of the adjacent teeth was negligible despite some slight palatal movement.
7.4 CLINICAL APPEARANCE

The five animals of the experimental group experienced marginal gingivitis around the experimental teeth. This appeared to resolve considerably upon removal of the orthodontic appliances. The presence of gingival disease can be attributed to infrequent tooth brushing, and to the food and plaque retaining potential of the orthodontic appliances.

Repeated appliance breakage and the development of periodontal disease are factors which can contribute to the success or failure of orthodontic treatment. Lindhe et al. (1973) worked with dogs that had markedly reduced periodontal support. They concluded that "in the dog, forces produced by occlusal trauma are unable to induce a phase of progressive destruction of the periodontal tissues in tooth regions where the supporting tissues are markedly reduced but non inflammatory". This finding was confirmed by Ericcson et al. (1978). This latter study demonstrated that the most important factor in the initiation, progression, and recurrence of periodontal disease, was the microbial plaque present within the gingival pockets. From our present study on beagle dogs, it was observed that, even with repeated appliance breakage and plaque and calculus accumulation, there was no evidence that the orthodontic extrusion technique lead to the development of debilitating advanced periodontal disease. However, the presence of plaque and calculus, in conjunction with the extrusive force placed on the tooth may have contributed to the development of cervical root resorption, by exposing the cervical root margin and allowing the ingress of bacteria. From a clinical perspective it is imperative that meticulous oral hygiene be performed during the extrusion period to lessen the incidence of inflammatory periodontal disease.

Svanberg (1973) observed that orthodontic forces were not capable of causing injuries to the supra-alveolar periodontal tissues in animals with normal gingiva or superficial gingivitis. The orthodontic brackets in position caused the accumulation of plaque that
lead to the development of mild inflammatory disease. This was in agreement with the studies of Lindhe et al. (1973) who found that the placement of orthodontic appliances was not a major factor in the development of periodontal disease. Simon et al. (1980) in his study on the root extrusion of premolars in dogs found clinically, the presence of gingivitis, the loss of gingival margin contour, and debris attached to the orthodontic appliances. He reported extreme mobility of the extruded teeth. This was a feature not observed in the present study.

7.5 RADIOGRAPHIC APPEARANCE

The most noticeable radiographic feature associated with the extruded teeth was the presence of a widened lamina dura at the completion of the active extrusion phase. This indicated orthodontic movement of the experimental teeth. This periapical rarefaction, was present after the active extrusion phase but was observed to have resolved by the time of sacrifice. A widened lamina dura was also observed in some of the adjacent anchor teeth. This indicated that those teeth underwent orthodontic movement also. Decrease of the periapical ligament width upon sacrifice indicated radiographic deposition of bone. The results obtained in the present study were in agreement with the results of Simon et al. (1980) who found that the root extrusion procedure was successful radiographically. Simon et al. (1980) found that radiolucent areas seen during the extrusion period appeared normal by the fourth week after extrusion.

7.6 EXTRUSION AND RELAPSE

One of the technical problems encountered with this project was the lack of implanted radiographic markers to determine the true extrusive movement of the experimental teeth. Consequently the only way of determining if actual movement of the experimental teeth had occurred was to devise a method of measuring the amount of extrusion relative to the adjacent incisor teeth. The use of the study models and brass points allowed a measurement of the relative extrusion of the experimental teeth.
technique was considered valid as the intrusive movement of the adjacent teeth has been difficult to achieve, and past authors have agreed that the initial orthodontic movement will always be extrusive (Stern and Becker, 1980). In orthodontic movement the least amount of force is required for extrusive movement, and the greatest force is required for intrusive movement (Simon, 1984).

Simon (1984) has stated that improper design and placement of the extrusion device may result in tipping of some abutment teeth. From the present study it was observed that movement of the adjacent central incisor teeth in some of the animals had occurred. This feature was confirmed radiographically. Movement of the adjacent teeth was most likely caused by improper design of the archwire or poor arch form.

From the results obtained, it was noticed that all five experimental teeth experienced orthodontic extrusion following the active extrusion period. Four of the five experimental teeth experienced extrusion of at least 1.1mm. The extrusive force used for these teeth varied from 30 to 120 grams. No relationship could be found between the force used and the amount of extrusion, and the small sample used precluded a statistical analysis of the results. Experimental tooth number five, which had the greatest initial extrusive force of 120 grams had the least amount of initial orthodontic extrusion. Thus it appeared that the initial extrusive force had no bearing on the actual amount of extrusion. Cooke and Scheer (1980) reported that any force less than 50 grams achieved little if any extrusive tooth movement. This contrasted with the present study in which orthodontic extrusion was found to occur with forces as low as 30 grams in animal number one.

All of the experimental teeth except tooth number five experienced relapse at the completion of the treatment period. Experimental teeth one, two, and four experienced the greatest amount of relapse. An interesting finding was that experimental tooth five had continued eruption after completion of the periodontal
surgery procedure. This was the only tooth to undergo a periodontal surgery procedure, and it was considered by the present authors that the severing of the gingival fibres may have allowed continued extrusive movement of the tooth. Experimental teeth one and four had the least amount of initial extrusive force, 30 and 80 grams respectively. There appeared to be a trend that the teeth with the least initial extrusive force have the greatest amount of relapse. This was not statistically quantified due to the small sample size.

Simon (1984) reported that "a slow steady force gradually raises the root to a position above the bone and gingiva in approximately one to three weeks", although no mention was made of the extrusive force to be used, nor was there any mention of relapse. In the present study all of the experimental teeth achieved extrusive movement within four weeks.

As only one animal underwent a periodontal surgical procedure it was difficult to conclude that this particular aspect of the technique was successful, although it did appear that the result obtained by this procedure agreed with the results of Brain (1969), that periodontal surgery procedure may play a role in reducing the amount of relapse of the extruded teeth.

7.7 GINGIVAL MARGIN ALTERATIONS
The gingival margin was found to move with three of the experimental teeth. In experimental teeth one and four, the gingival margin maintained a constant distance with the experimental teeth as they underwent extrusion. These two teeth had the least initial extrusive force. In experimental tooth two the gingival margin moved a portion of the extrusion distance. With experimental tooth three the gingival margin was found to maintain its original position. In experimental tooth five the gingival margin moved away from its original position by 0.4mm. It appeared that there was a trend for the gingival margin to move with the tooth when a lighter force of extrusion was
used. This was not statistically quantified due to the small sample size. The result achieved by experimental tooth five was most likely assisted by the tissue reduction performed during the periodontal surgery procedure. Simon et al. (1978) considered that movement of the gingival margin was a function of how rapidly the tooth was extruded and how much extrusive force was used. He observed clinically in patients that the gingival unit or housing, may or may not move occlusally with the extruded tooth. Simon (1984) also observed that the teeth extruded with heavier forces had a decreased tendency for movement of the gingival margin. Simon (1984) also reported that "to have the gingival housing move occlusally with the tooth the extrusion time is longer and the force applied is less". Potashnick and Rosenberg (1982) found that a lag occurred between the movement of a tooth and movement of its gingival margin attachment apparatus. The amount of force used and the speed of eruption determined the lag time. The faster the tooth was erupted forcibly from the alveolus the greater will be the lag between the movement of the tooth and its attachment apparatus. The findings in the current study appear to support the findings of Potashnick and Rosenberg (1982) where the tendency for relapse of orthodontically moved teeth and movement of the gingival margin was decreased with the use of heavier forces.

Simon et al. (1978), Simon (1984), and Heithersay (1973) considered that a surgical procedure may be required following the extrusion of a crown-root fractured tooth. McDonald et al. (1982) found it necessary to use a full thickness mucoperiosteal flap with an internal bevel incision to reposition the gingival margin on the labial aspect of an extruded tooth. Kozlovsky et al. (1988) developed a technique where incisions of the supracrestal gingival attachment were performed to prevent reintrusion and movement of the gingival margin during the extrusion procedure. These authors considered that the technique was extremely successful and avoided the need for osseous surgery.
Wong and Fricker (1991) performed a periodontal surgery procedure on the gingival margin of a twelve year old girl who underwent a root extrusion procedure. They performed a reverse bevel palatal flap in conjunction with a gingivectomy, and reported complete success and healing of the soft tissues and no relapse of the extruded tooth. There was no mention of continued eruption of the root filled extruded tooth. The success of the periodontal surgery procedure has been confirmed clinically by other authors including Biggerstaff et al. (1986). Biggerstaff et al. (1986) noticed that forced eruption caused a disparity between the epithelial attachments between the extruded and adjacent teeth. They observed that when this situation was corrected surgically the gingiva was repositioned and the epithelial attachment levels were restored.

The periodontal surgery procedure used in this present study was successful in preventing relapse and providing access to the decoronated crown margins. As this procedure was performed on only one tooth further investigation is required to determine the long term success of this technique.

No long term studies on the use of periodontal surgery procedures have been performed on extrusive movements of teeth. Most of the clinical studies have been performed on rotational movements of teeth. From the single animal that underwent a periodontal surgery procedure in this study it appeared that a surgical procedure may be beneficial towards reducing the amount of orthodontic relapse.

7.8 SCANNING ELECTRON AND LIGHT MICROSCOPE FINDINGS

Great variation was observed for periodontal ligament width; cementum thickness; blood vessel size, number, and vascular area for the experimental and control teeth. Analysis of the experimental teeth involved a comparison between the control teeth and also between the non root filled second lateral incisor.
7.8.1 Periodontal Ligament Thickness

Very little has been written concerning the effects of orthodontic extrusion upon the periodontal ligament. Research has determined that the width of the periodontal ligament may vary throughout the length of each tooth for humans and experimental animals including rats and dogs (Berkovitz, 1990). From the present study it was observed that the width of the periodontal ligament was slightly greater for the experimental teeth than the control teeth. By contrast the width of the periodontal ligament of the non root filled teeth appeared similar to that of the root filled teeth. A noticeable feature in one of the control teeth (number six) was a decreased periodontal ligament width towards the apex of the tooth. The width reduced from 269μm at the cervical region to 81μm at the apical region of the tooth. Crawford et al. (1986) observed that, in unopposed molars which underwent extrusive movement, there was a trend for a decreased periodontal ligament width. They concluded that this was due to a nett effect of masticatory hypofunction. Crawford et al. (1986) found that the vascular supply to the teeth decreased, and consequently the nutrient supply to the teeth was also decreased. As the control teeth in the present study were decoronated, they were in hypofunction, and consequently unable to function as an incisor of a normal length. Hypofunction of the control tooth may have explained the similar observations to those reported by Crawford et al. (1986). The unusual feature associated with the control tooth in the present project that illustrated narrowing of the periodontal ligament was that the mid root and apical measurements were similar. The difference was related to the extreme width of the periodontal ligament cervically. There may also have been some widening of the cervical periodontal ligament which exacerbated the result. The experimental teeth in the present project did not show a decrease in periodontal ligament width apically. As these teeth underwent orthodontic extrusion it can be assumed that they were still undergoing biological adaptations to the orthodontic forces. Consequently the experimental teeth could not be considered hypofunctional. Control tooth number seven had a generalised decrease in width compared with the other six teeth. This may have been due to anatomical reasons.
7.8.2 Cementum Alterations

Cementum has a capacity to adapt to areas of tension and stress (Genco et al., 1990; Tronstad, 1988). It has been shown that cellular cementum can be deposited as a tooth undergoes movement and resorption (Tronstad, 1988). Simon et al. (1980) also noticed the deposition of cementum towards the apex of extruded teeth in dogs. The results of the present study confirmed the deposition of cellular cementum towards the apex of the experimental teeth. It was surprising to observe cementum deposition towards the mid root and apex of the control teeth. The finding of cementum deposition towards the apex of the control teeth may confirm the previous consideration (Crawford et al., 1986) that after decoronation the tooth attempted to return to a physiologically normal functioning position. Compared to the non root filled adjacent teeth the amount of cementum deposition was considerable. In animals one to seven there was a increase of cementum towards the apex of the root filled teeth. The thickness of the cementum deposition was greater for all five experimental teeth than their adjacent non root filled second lateral incisors. One would have expected to find increased cementum thickness of the extruded teeth only, yet as the decoronated control teeth may have had continued eruption, further deposition of cementum may have occurred. This feature requires further consideration and research.

7.9 VASCULAR ALTERATIONS

The most obvious histological and SEM observations involved alterations to the vascularity of the extruded teeth. Before proceeding it must be restated that examination of the vascular alterations was restricted to a small area of the interproximal region between the root filled teeth and the second lateral incisor only. Rygh et al. (1986) in a study on the movement of molars in rats for two, seven, fourteen, and twenty eight days with a force of thirty grams found considerable alterations to the vascular system. By moving a rat molar mesially they found the following:
i. An increased number of blood vessels adjacent to and an invasion of vascular structures into compressed hyalinised areas.

ii. An increased blood supply to areas of osteoclastic activity.

iii. An increased vascularisation in areas of tension.

Their evidence also indicated that the degree of vascular activity was dependant on the amount of stress to which the periodontal ligament was exposed. These authors also found that in areas of tension, an increase of vascular volume at the expense of fibrillar volume was observed after one week at all levels of sectioning throughout the specimens.

Lew et al. (1989) found that in an extruded rat molar for thirty minutes that the mean vascular volume, as a percentage of periapical ligament volume increased in post capillary sized venules (from 16.6% to 22.3%), venous capillaries (from 2.0% to 2.7%), arterial capillaries (from 0.4% to 1.0%), and terminal arterioles (from from 1.0% to 2.5%). Kvinnsland et al. (1989) found a substantial increase in blood flow of experimentally moved teeth in rats after five days. It has become apparent that short term alterations to the vasculature are related to a localised inflammatory reaction, as evidenced by red blood cell diapedesis (Cooper and Sims, 1989). The present study indicated alterations to the vasculature in terms of vessel size and total vascular area. The changes present were on a long term basis because they were still present up to three months after the extrusion. Weir and Sims (1991) observed that after incisor extrusion of 1.2mm in marmosets of, and long term retention of thirty weeks, reconstitution of the periodontal vascular bed was present. They found no statistical difference between the control teeth and the experimental teeth. In the present study there vascular alterations (blood vessel area, diameter, and number) between the extruded teeth and the control teeth. This was not statistically quantified due to the small sample size. In comparing the vascular areas of the root filled teeth and their adjacent non root filled counterparts, there was a similarity between four of
the seven teeth. There was a noticeable increase in vascular area between the experimental teeth and the adjacent teeth in animals one, two and four.

7.9.1 Blood Vessel Numbers

Results indicated that the extruded teeth did not have an increase in total blood vessel number compared with the control teeth. There appeared to be an overall increase in mean blood vessel diameter for three of the experimental teeth when compared with the control teeth (Graph 6-7). The blood vessel diameter of the root filled teeth appeared to be greater than the non root filled teeth. Overall no consistent trend in blood vessel diameter could be determined between the control teeth, experimental teeth, and adjacent non root filled incisor teeth. A study on orthodontic extrusion of teeth have observed that there is an increase in blood volume in tension areas following orthodontic extrusion (Batenhorst et al., 1974). With extrusive movement of teeth only tension is placed upon the root surface and an increase in vascularity can be expected. In the present study as a compensatory measure an increase in vessel size may have occurred rather than an increase in vessel number.

7.9.2 Blood Vessel Diameter

Blood vessel diameter was measured to determine if there were any differences between various regions of each root filled tooth. There was a trend for increasing mean blood vessel diameter towards the apex of three of the experimental teeth. The blood vessel diameter of the control teeth appeared to either remain constant (animal 7) or decrease in width from the cervical region to the apical region (animal 6, Graph 6-6). It appeared from measurement of blood vessel area that to compensate for the decrease in blood vessel numbers apically there has been a concomitant increase in size. The diameters of the blood vessels of three of the experimental teeth generally appeared greater than those of the control teeth. The mean blood vessel diameter for four of the experimental animals appeared greater than the adjacent non root filled lateral incisor. Khouw and Goldhaber (1970), concluded from their histological
observations, that augmented vascularity following orthodontic movement of teeth in
the monkey and dog was due to an increase in vessel diameters and not to an increase
in their number. As there was such great difference in periodontal ligament
vascularity between the experimental teeth it is difficult to determine whether the
results obtained in the present study confer with those of Khouw and Goldhaber. At
best it can be said that there is a degree of similarity.

7.9.3 Vascular Area

In two of the experimental teeth there was increasing vascular area towards the apex
of the tooth, whereas in the other three experimental teeth there was decreasing
vascular area for the length of the tooth. There was also a difference in vascular area
between the experimental root filled teeth and the non root filled second lateral
incisors. In four of the five experimental teeth the total vascular area was greater than
those of the adjacent non root filled teeth. It was apparent that the extrusion procedure
produced an increase in the blood vessel area by complex alterations of blood vessel
size, and number. Root canal therapy does not appear to have altered the total
vascular area as the vascular area for the two root filled control teeth was similar to
their adjacent non root filled second lateral incisors.

Steigman et al. (1991) found that, after two weeks of continuous loading on a rat
incisor the overall volume of the compressed periodontal ligament had decreased by
22% whereas the volume of the periodontal ligament under tension had increased by
72%. In their study there was an even greater change in the apical third of the tooth;
they observed an addition of 177% to the vascular volume of the tensioned
periodontal ligament and a 43% decrease in the vascular volume of the compressed
periodontal ligament. Even though these results were obtained from loading of rat
incisors they do confirm that major alterations occur to the periodontal ligament
vasculature following the application of tensional forces. Another interesting factor
observed by Steigman et al. (1991) was that the vascular volume of the tensioned
periodontal ligament was still 25% greater than the control teeth even ten weeks after the orthodontic forces were removed. The results obtained in this study observed that upon completion of the experimental period the vascular area of the periodontal ligament of three of the experimental teeth was greater than the control teeth.

In summary complex alterations to the periodontal ligament vasculature have occurred subsequent to the orthodontic extrusion procedures. An interesting feature observed in the present project is that after completion of the experimental period the vasculature in three of the extruded teeth did not return to values similar to the control teeth or the adjacent non root filled second lateral incisors.

7.10 RESORPTION

Cervical root resorption was present in three of the five experimental teeth. It was not present in the two control teeth. Cervical resorption has been considered to be caused by a number of factors including trauma, bleaching processes, and periodontal surgery (Tronstad, 1988). In many cases it has been seen as a late complication to traumatic injuries of the teeth (Tronstad, 1988). Tronstad (1988) in a clinical study of patients undergoing fixed orthodontic therapy, observed cervical resorption to be diagnosed in one incisor from eighty seven patients. He noted that it was a rare occurrence following orthodontic treatment and unlike apical resorption, which will halt following orthodontic treatment, cervical resorption can be sustained by infection and will therefore not stop when the orthodontic forces are removed. Extrusion or intrusion of teeth can result in traumatic damage to the root (Tronstad, 1988). This can result in denuded areas on the root surface which will be chemotactic to hard tissue resorbing cells. Root resorption can then ensue (Tronstad, 1988). It has been shown in monkeys that if the gingival epithelium can be prevented from regaining its original position then cervical resorption can develop. Brosjo et al. (1990) found that foil covered gingival margin cavities in teeth of monkeys promoted the development of cervical resorption in the presence of inflammation and plaque, because the surface
was devoid of epithelial coverage. Cervical resorption present in tooth number five was the severest of the experimental group. This was the only tooth to have undergone periodontal surgery. The most likely cause of cervical root resorption in experimental tooth number five was a combination of factors including poor gingival health, heavy forces of extrusion, and trauma caused by the animals masticatory habits, which included chewing on cage bars, which damaged their orthodontic appliances. The finding of cervical resorption was consistent with the findings of Batenhorst et al. (1974) who observed resorption of cementum and dentine of teeth following the extrusive movement of mandibular incisors in two adult rhesus monkeys. This finding of cervical resorption was a significant feature in the current project because it was a detrimental factor to the extrusion procedure. However, at the completion of the experimental period, histological sectioning did illustrate that cementum repair of the resorptive areas did occur. Osteoclasts and cementoclasts were not observed histologically indicating that resorption had occurred prior to, and was complete at the time of sacrifice.

Artun and Aamda (1987) described a clinical case in which cervical resorption was observed twelve months following the orthodontic extrusion of a root filled incisor. No cause for the cervical resorption present was found as it was noticed only after the orthodontic appliances were removed. Wong and Fricker (1991) did not detect clinically the presence of cervical root resorption in a patient they had treated for orthodontic root extrusion.

No evidence of root resorption was observed in the apical or mid root regions in any of the control or experimental teeth. Simon et al. (1980) observed the absence of root resorption in dog premolar teeth following extrusion. They did not mention the presence or absence of cervical root resorption. A clinical study by Spurrier et al. (1990) on contralateral premolar teeth to be extracted for orthodontic purposes reported that the resorption of root filled teeth following orthodontic treatment was
statistically less than for vital teeth. Clinically it was found to have no bearing on the treatment outcome for the patient.

In conclusion, the observation of cervical root resorption in three of the experimental teeth was a significant feature in the present study. This was the most detrimental feature observed for the entire project. Although the resorption present appeared to be histologically arrested, further investigation is required to determine the exact cause of the resorption and the exact time of its occurrence.

7.11 CELLULAR CONTENT

To explain the soft tissue changes that occur following orthodontic tooth movement, Sicher (1942) introduced the concept of the "intermediate/indifferent fibre plexus". As previously reviewed in chapter four, many authors consider that the intermediate plexus is an artefact of tissue processing. Ten Cate et al. (1976) considered that the fibroblast was capable of generating and degenerating collagen and it was this cell which performed a significant role in the remodelling of the periodontal ligament. The majority of cells determined by histological analysis, within the periodontal ligament of the animals in the present study, were fibroblasts. No evidence of inflammatory cells were found throughout the length of the experimental or control teeth.

In the present study, Sharpey's fibres could be clearly seen passing from tooth to alveolar bone, and throughout the periodontal ligament. No evidence of an "intermediate plexus" could be located as histological sections illustrated an orderly arrangement of periodontal ligament fibres and fibroblasts. Oxytalan fibres were not examined for, as special staining techniques were not employed to look for them. The most characteristic feature of the periodontal ligament was that the fibres of the ligament appeared to be arranged normally. Unusual elongation or stretching of the fibres was not observed in either longitudinal or horizontal sections. This finding conforms with the study by Simon et al. (1980) who found that the fibres of the
periodontal ligament of extruded premolar dog teeth had reorganised themselves by the time the animals were sacrificed. Also, very few plump fibroblasts were present in the present project indicating that there was not a high turnover of collagen (Ten Cate et al., 1976).

When discussion of the collagenous fibre bundles of the periodontal ligament has appeared in the dental literature, one observation has been consistent, "the supracrestal/supra-alveolar connective tissue fibres, whose length and direction are not altered by an ever changing osseous attachment are extremely slow to adjust to orthodontic movements of the teeth" (Ten Cate et al., 1976; Edwards, 1968).

Ten Cate et al. (1976) reported that physiologic activity, namely fibroblastic activity, can slowly, from six to twelve months remodel the fibres of the supracrestal region. This was supported by the findings of Edwards (1968) who observed after rotational movements of teeth that only "after five months of retention the fibrous bundles of the periodontal ligament, as well as the transeptal fibres closest to the alveolar crest appeared completely adapted to the new rotational position of the tooth". Edwards (1968) found that the supracrestal fibres of the periodontal ligament were the slowest to remodel following orthodontic movement of teeth, which agrees with the previous quote from Ten Cate et al. (1976). The finding of normal periodontal ligament fibre orientation, following the extrusion of incisor teeth of beagle dogs in the present study appear to lend support to the findings of Edwards (1968), and Ten Cate et al. (1976), that fibre remodelling can occur although extremely slowly after a sufficient period of retention. The limited amount of relapse in animal five following the periodontal surgery procedure performed on it indicates that periodontal surgery may have a role to play towards decreasing relapse.

Rygh et al. (1986) in their experiments on movement of mouse molars found that in areas of tension after one week, there were numerous macrophages around the blood
vessels, indicating the presence of an inflammatory process. This finding was confirmed by Cooper and Sims (1989) who noted red blood cell diapedesis after extrusive movement on a mouse molar for thirty minutes. A feature of the present study was the absence of inflammatory cells. Even though gingival inflammation was observed clinically, inflammatory cells were not detected. This most likely is due to the regions examined, as the gingival tissues were not always intact when histologically examined. Processing error may have also contributed to this result. Also the present study involved examination of specimens after many months of experimental observation, and deeper into the periodontal ligament the initial inflammatory process would have resolved. The previous reports of Rygh et al. (1986), and Cooper and Sims (1989) were only short term studies, and the results they achieved perhaps represented a normal inflammatory response of the tissues.

7.12 VESSEL CLASSIFICATION

Histological observation observed that the majority of the vessels in the experimental animals were venular in appearance, only occasional arterial vessels were observed. The presence of venules was determined by their cell wall appearance and the absence of elastic fibres within the cell wall. A predominance of venular structures appeared to correlate with the results of Weekes (1983) who observed very few arterial elements in the vascular bed of the rat periodontal ligament.

The periodontal ligament of the dog was reported to have arterioles coursing within it (Ichikawa et al., 1976; Kishi and Takahashi, 1977). These arterioles supplied a capillary plexus and did not run for any great distance in the ligament. The exception to this was the coronal quarter of the ligament where circularly orientated bundles of thick vessels existed each containing a venule and one or two arterioles. In the present project vascular bundles consisting of large venular blood vessels and an occasional arteriole were observed. The larger vessels of the extruded experimental teeth appeared to be situated towards the outer one third of the periodontal ligament,
that is, closer to the alveolar bone than to the cementum. Histological observation confirmed that the majority of the vessels examined in the present study of the beagle dog periodontal ligament were venular in origin.

Vascular bundles containing neural elements were almost absent in the periodontal ligament of the experimental teeth. Neural supply to the adjacent non root filled lateral incisor periodontal ligament appeared to be greater than for the experimental teeth. This observation was not quantified, and was based on visual observation of the histological specimens only. Weir and Sims (1991) detected the presence of a reconstituted neural supply to the periodontal ligament of a marmoset incisor following root extrusion and retention.

7.13 ALVEOLAR BONE HEIGHT

Biggerstaff et al. (1986) confirmed clinically that root extrusion procedures consistently lead to apposition of bone, and these authors claimed that one, two, and three walled periodontal defects may be resolved in this manner. Melsen (1986) in a study on monkeys found that following extrusion of teeth, alveolar bone height was increased. Heithersay (1973) and Simon et al. (1980) have also previously reported the deposition of bone at the alveolar crest and throughout the length of the tooth following orthodontic extrusion of the teeth. Histological sections observed the deposition of alveolar bone adjacent to experimental tooth number five only. This was unable to be confirmed by longitudinal sections. Insufficient retention of hard tissue during the specimen preparation procedures may have lead to an inability to examine the deposition of alveolar bone in the remaining specimens. Radiographically, apical bone deposition was observed in all five experimental teeth prior to sacrifice. Further investigation into the deposition of alveolar bone is required, as the present project does not provide sufficient information relating to alveolar bone deposition following extrusion. Defleshing of the specimens may provide an alternative to histological examination. In this manner alveolar bone
deposition may be observed around the tooth, and compared with the bone height of the adjacent teeth.

7.14 GENERAL DISCUSSION

The most notable feature when compiling the literature review for this topic was how little has been documented concerning the orthodontic extrusion of non vital root filled teeth. Other than the histological study performed on decoronated-root filled extruded teeth in dogs by Simon et al. (1980) very little has been documented on this subject. The most recent study was by Weir and Sims (1991), who examined the extrusion of root filled incisor teeth in marmosets.

Crown-root fractured teeth have always presented a difficult treatment challenge for the paediatric dentist. A number of treatment modalities have been presented to restore and to treat the crown-root fractured tooth. A general review of the literature illustrated that the numerous treatment alternatives for extrusion of the crown-root fractured tooth have been based on empirical findings only. Empirical clinical experience has suggested that root fragments can be extruded three to four millimetres in one to six weeks (Heithersay, 1973; Wolfson and Seiden, 1975; Simon et al., 1978; Stern and Becker, 1980; Simon, 1984). In general, retention periods determined by the above authors ranged from eight to twelve weeks. The present study observed that extrusion could be achieved in the five experimental animals within four to six weeks. The amount of extrusion obtained was similar for all five animals independent of the initial extrusive force utilised.

Simon et al. (1980) and more recently Weir and Sims (1991) have been the only authors to publish extensive animals studies restricted to the orthodontic root extrusion technique for crown-root fractured root filled teeth. The use of the beagle dog in the present study proved to be difficult and illustrated that the method of
extrusion and retention with directly bonded orthodontic appliances require greater thought and consideration. The technique of utilising cemented cast cap splints, advocated by Simon et al. (1980) appeared to be fraught with less problems.

An interesting feature of the present study was the introduction of the upper right second lateral incisor as a second comparative parameter. This was performed in an attempt to determine if root canal therapy had any effect upon the healing properties of the periodontium, and also to determine if alterations to the periodontal ligament vasculature differed between root filled and non root filled vital teeth. It appeared that the changes to the periodontal ligament vasculature were attributable to the extrusion procedure, as illustrated by the difference in blood vessel area between the root filled teeth and the non root filled teeth, and the control teeth. Also, alterations to the cementum thickness, and periodontal ligament thickness of the non root filled teeth differed little to the control teeth, indicating that most of the changes experienced by the experimental teeth were caused by the extrusion procedure.

Cervical root resorption was a significant feature observed in the extruded teeth, and this problem requires further investigation. It is necessary to determine the time of onset for cervical resorption, whether it was during the extrusion phase, retention phase, or the waiting period. It would also be useful to determine the duration of active cervical resorption, and also to determine the aetiological factors involved. Cervical resorption was observed to be present in the teeth that underwent extrusion with heavier forces.

The results from this study indicated that lighter forces of extrusion, less than 80 grams, favour relapse of the extruded tooth. Lighter forces of extrusion also favour movement of the gingival margin. The present study demonstrated that heavier forces of extrusion favoured less movement of the gingival housing with the extruded tooth, confirming observations by Simon (1984).
Further research into the effects of orthodontic movement of vital and non vital teeth is necessary as insufficient material has been documented concerning the difference between the two. Further investigation into the role of the fibroblast is required as this seemed to be the predominant cell accounting for the soft tissue remodelling phase. The orthodontic movement of teeth is a complex phenomenon that requires further research. Orthodontic root extrusion is a complex procedure, and the present study has illustrated that in the beagle dog it can lead to long term vascular alterations and reorganisation of the periodontal ligament fibres.
8 CONCLUSIONS

1. No previous study has examined the extrusion of decoronated root filled teeth of a beagle dog utilising conventional bonded Beddtiot orthodontic appliances. The use of such appliances is advantageous for extrapolating results to the human subject.

2. The technical procedures utilised throughout this study proved to have a number of complications. Improvements to the orthodontic appliances will be necessary to ensure adequate retention, and consistent distribution of orthodontic force. Modification of the extrusion device may be necessary for future research as problems with appliance breakage were experienced in the present study. Future research will require further attention to techniques of perfusion and fixation, and histological processing of specimens.

3. Root canal therapy itself appeared to minimal effect on the morphological changes observed in the periodontal ligament. The extrusion procedure itself appeared to play a dominant role in the morphological alterations observed. This was noticeable when the root filled teeth as a group were compared with the non root filled second lateral incisor teeth, and the two control teeth.

4. Radiographs confirmed excellent healing of the periodontal ligament of the root filled extruded teeth.

5. Extrusion was achieved with both light and heavy forces. Relapse was experienced with four of the five experimental animals, although a greater tendency for relapse existed when lighter forces of extrusion (< 80 grams) were utilised.

6. The gingival margin was noted to move with the tooth when lighter forces of extrusion were utilised.

7. The predominant cell within the periodontal ligament was the fibroblast.

8. Cervical resorption was a detrimental factor affecting the extrusion procedure. It was noticed in three of the five experimental animals. These three animals had forces of extrusion greater than eighty grams. One of these three animals underwent a
periodontal surgical procedure. Histologically the resorption was arrested, with no evidence of cementoclastic cells.

9. The fibres of the periodontal ligament appeared to orient themselves to a normal appearance on completion of the experimental period. The architecture and appearance of the periodontal ligament fibres of both the control teeth and experimental teeth appeared similar.

10. Alterations in periodontal ligament vascularity involving increased blood vessel diameter, and an increase in vascular area for the experimental teeth, were observed within the periodontal ligament. Further investigation into the vascular alterations following orthodontic tooth movement of teeth is required to provide greater understanding of the biological alterations within the periodontal ligament subsequent to orthodontic root extrusion.

11. Due to the small number of teeth available for examination, no definitive statistical analysis was performed. This project has provided a basis for further investigation into the orthodontic extrusion technique, and for investigation of the orthodontic movement of non vital teeth.
8.1 CLINICAL RECOMMENDATIONS

From the results obtained in the current project and a review of previous literature the following clinical recommendations for the extrusion of crown-root fractured teeth have been developed.

1. Orthodontic root extrusion should be accomplished with meticulous oral hygiene.
2. Light forces of extrusion should preferably be utilised (less than 80 grams) to decrease the possibility of cervical root resorption developing.
3. To overcome the possibility of relapse associated with the lighter forces of extrusion, a longer retention period may be utilised. From the reports already published on relapse of rotated teeth and the duration of the current project this should be six months. Long term retention may allow reorganisation of the supra-alveolar fibres, which appear to be the main contributing factor to the relapse of orthodontically moved teeth.
4. Pericision or a periodontal surgical procedure may be a beneficial factor, both preventing relapse and enabling exposure of the crown margin. However care must be taken when performing this procedure as aggressive curettage may initiate cervical resorption.
5. The duration of active extrusion should be dependant on the distance the root fragment is required to be extruded.
6. Regular radiographs and careful clinical examination need to be initiated to detect any evidence of root resorption, periodontal disease or excessive tooth mobility.
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ZWARCH PD, QUIGLEY MB (1965)
10 APPENDICES

APPENDIX 1

Washout Media

For each animal 1.5L of washout media was introduced to the animals prior to fixation.

The washout media consisted of:

i. Sodium Chloride (Ajax Chemicals. Sydney, Aust.)
   9.0 grams for every 1.0L of water.

ii. Heparin (Heparin Sodium injection BP Mucous. Glaxo Australia Pty Ltd)
   1.0mL of 1000IU/mL heparin for every 100.0mL of water to make a concentration of 10IU/mL.

iii. Papaverine HCl (120mg/10.0mL, David Bull Lab Pty Ltd.)
    0.1mL of papaverine for every one litre of water.

iv. Polyvinylpyrrolidone, MW 40,000 (PVP 40, Sigma Pty Ltd.)
    58.74 gm for every 1.0L of water which provides a colloid blood pressure of 25mm Hg.

The medium was mixed in an aspirating bottle on a heated stirrer until a temperature of forty five to fifty degrees centigrade was achieved.
APPENDIX 2

FIXATIVE

1. Methanol Free Formaldehyde

1.5L of fixative was introduced to each animal following vascular washout.

i. Paraformaldehyde

120g dissolved in 1500mL of tap water preheated to 60°C.

ii. 10N NaOH added dropwise to clear the precipitate.

iii. pH adjusted to 7.4 with NaOH.

iv. Equal volume of 0.1M phosphate buffer, pH 7.4 was added. Stored at 4°C

2. Phosphate buffer (Sorensens)

A. NaH₂PO₄ 2H₂O

MW: 156.01

1M=156g/L

B. Na₂HPO₄

MW 141.96

1M=141.96g/L

0.2M of stock solution was required of both A and B.

To make 2L of buffer 0.1M 1600.6mL of B was mixed with 399.2mL of A.
APPENDIX 3
The following illustration is a copy of the original tracing used to measure the amount of extrusion of the experimental teeth, and also movement of the gingival margin. This tracing is only for four of the animals, and is presented to allow a thorough understanding of the technique employed to measure the amount of extrusion experienced by the experimental teeth. As listed in chapter five, radiographs were taken of the study models with brass points in position. Tracing paper was placed over these radiographs, and movement of the gingival margin, and the decoronated edge of the experimental teeth were measured relative to a straight line drawn between two adjacent teeth.

Appendix 3 1: represents the pre-extrusion radiograph, 2: represents the post extrusion radiograph, 3: represents the post retention period, 4: represents the post waiting period, pre sacrifice period. The top X represents the incisal edge of the tooth, the lower X represents the gingival margin.
APPENDIX 4

Raw data for the seven animals that underwent root canal therapy is presented here. For the following tables:

RCT represents the root filled experimental or control tooth.
ADJ represents the adjacent non root filled second lateral incisor.
SD represents the standard deviation
S.E.M represents the standard error of the mean.

**Beagle 1**

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<th>RCT</th>
<th>ADJ</th>
<th>RCT</th>
<th>ADJ</th>
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<td>PDL thickness (µm) mean values</td>
<td>Cementum thickness (µm) mean values</td>
<td>Blood vessel diameter (µm) mean value (N) number of blood vessels</td>
<td>Total vascular area for specified region (µm²)</td>
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