BIOLOGICAL RESPONSE TO CORTICOTOMY-ASSISTED ORTHODONTICS

A thesis submitted in partial fulfilment of the requirements for the degree of
Doctor of Clinical Dentistry (Orthodontics)

by

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3. SIGNED STATEMENT

This report contains no new material that has been accepted for the award of any other degree or diploma in any other university. To the best of my knowledge and belief, it contains no material previously published except where due reference is made in the text. I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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4. SUMMARY

The aims of this study were to validate the local and regional biological response to pre-orthodontic corticotomy in an animal model; and to evaluate the local and regional bone response to a flap or corticotomy procedure with and without tooth movement.

Thirty-six Sprague-Dawley rats, six to eight weeks old, were divided into six groups and treated over a seven-day period.

Group	Intervention
1	Nil
2	Flap only
3	Corticotomy only
4	Tooth movement only
5	Tooth movement and flap
6	Tooth movement and corticotomy

All treatment procedures were performed on the right maxillary first molar. A custom appliance was placed at the start of the observation period and surgical procedures were performed at time of placement. In the surgery groups, a full-thickness flap was elevated along the buccal surface of the right maxillary first molar and a distal vertical relieving incision between the roots of the first and second maxillary molars.

For the corticotomy groups, a slow-speed hand piece and a 0.5 mm round bur was utilised to create a trench through the cortical bone extending from the apices of the first molar horizontally and mesially beyond the mesial root in an L shape. A fixed appliance with 100 g NiTi spring was placed to produce buccal tooth movement over a total experimental time of 7 days. Following sacrifice, the specimens were prepared and resin-embedded.

Micro-CT scans of the samples were performed utilising a Skyscan 1174. CTan (CT analysis program) was used to measure the bone mineral density (BMD) and bone fraction of a defined region of interest. The buccal and palatal plates of bone were assessed to evaluate local and regional bony changes, respectively.

Three-dimensional images of the samples were reconstructed using the Paraview program and macroscopic differences within the buccal region of bone between treatment groups could be seen in the reconstructed images, with the greatest changes visible in the combined 'Tooth Movement and Corticotomy' group. Similar osseous changes were visible in the untreated region of palatal bone in the same group.

After seven days of treatment, the BMD of the buccal region of bone on the treatment side tended to decrease relative to controls, although the differences were only statistically significant for the combined 'Tooth Movement and Corticotomy' group.

Comparison of the buccal region on the treatment side of all groups showed a pattern of change in bone fraction consistent with the level of intervention involved. The bone fraction of the 'Tooth Movement and Corticotomy' group was statistically significantly reduced compared with all groups except for the 'Tooth Movement and Flap' group. In the assessment of the palatal regions, variable results were found when the control side was compared with the test side.

In conclusion, following corticotomy and seven days of buccal orthodontic tooth movement in the rat model, there was a significant reduction in bone volume fraction in the buccal region of bone. This suggests that, in an animal model, corticotomy combined with orthodontics was able to accelerate the bone resorption and formation processes associated with tooth movement, which supports the clinical results observed in previous reports of corticotomy-assisted orthodontics. Furthermore, there were osseous changes in the palatal region of bone, suggesting that there is a regional and systemic regional acceleratory phenomenon effect.

5. LITERATURE REVIEW

Introduction

Orthodontic treatment involves the movement of teeth through alveolar bone using externally applied forces and results in a biological reaction within the dento-alveolar tissues. Hence, orthodontics is characterised as bone manipulation therapy and it is the biomechanical manipulation of bone that is the physiologic basis of orthodontics and facial orthopaedics. The remodelling of bone following injury or stimulus involves a complex array of interwoven processes and these ultimately determine the rate of tooth movement. Hence, there is a limitation to the rapidity at which orthodontic treatment can be completed without adverse effects. Limitations of traditional orthodontic techniques and the length of requisite treatment times often result in difficulties for providers and create barriers to patient willingness to accept orthodontic care. Several adjunctive treatment modalities have been examined in order to accelerate orthodontic tooth movement, including pre-orthodontic corticotomy (1-5).

Corticotomy-facilitated orthodontic therapy can appreciably expand the boundaries of orthodontics and greatly shorten treatment times. Corticotomy cuts or selective alveolar decortication (SAD) induce therapeutic “trauma” causing bone demineralisation through the regional acceleratory phenomenon (RAP) (6) that “supercharges” dentoalveolar tooth movement.

Bone

Bone is a living tissue and, being a connective tissue, consists of cells and an extracellular organic matrix. It is a metabolically active tissue that is capable of responding to changes in mechanical stimuli, adapting its internal architecture, and repairing structural damage. The processes of bone formation and resorption are regulated through the actions of the bone cells: the osteoblast and osteoclast. The formation of the organic matrix of bone, which consists of collagen fibres in a mucopolysaccharide-rich, semi-solid gel (ground substance), is executed by the osteoblast. This extracellular matrix becomes calcified through the precipitation of calcium phosphate crystals within the matrix and, during this process, the osteoblast becomes entombed in the deposited matrix, becoming an osteocyte. The osteocytes, which are no longer involved in new bone-forming duties, are responsible for maintaining the bone tissue and participate in the calcium exchange between bone and blood. Osteoclasts are responsible for bone resorption and are regulated by osteoblasts (and derivatives) through a signalling axis that is able to control osteoclast generation and activity (7).

Orthodontic tooth movement

Orthodontic tooth movement, defined as the 'result of a biological response to interference in the physiological equilibrium of the dentofacial complex by an externally applied force' (8), involves an integrated array of bone modelling and remodelling events (9). The site-specific remodelling is mediated by physical, cellular, biochemical, and molecular reactions taking place in the periodontal tissues, which constitute a coupled process of bone resorption and bone formation (10).

Theories of orthodontic mechanisms

There are several proposed mechanisms for tooth movement, including the more popular "pressure-tension theory" and the bending of the alveolar bone, which is preferred by the bone biologists.

Pressure-tension theory

Oppenheim (11) and Schwarz (12) hypothesised that a tooth moves within the periodontal space by generating a "pressure side" and a "tension side". The pressure side, located in the direction of tooth movement, displays disorganisation and diminution of fibre production. Additionally, the associated vascular constriction results in a decrease in cell replication. Conversely, on the tension side, there is stimulation of processes with increased cell replication and fibre production (13). This has been attributed to the stretching of the periodontal ligament fibre bundles.

As a consequence of the forces exerted on the 'pressure side', the inflammation results in cellular recruitment and tissue remodelling. This in turn leads to the activation of frontal resorption and undermining resorption. There is resulting loss of bone mass at pressure areas and apposition at tension areas (14).

Additionally, there may be hyalinization generated in the adjacent marrow spaces. The first sign of hyalinization is the presence of pyknotic nuclei in cells, followed by the appearance of areas of acellularity or cell-free zones. These changes have been proposed to occur as a result of width changes in the periodontal ligament space, changes in cell population, and increases in cellular activity leading to disruption of collagen fibres coupled with cell and tissue damage (15).

Cellular elements such as macrophages, foreign body giant cells, and osteoclasts from adjacent undamaged areas invade the necrotic tissue to resolve the inflammation in the area. The process of undermining resorption occurs as a result of the cells removing the necrotic tissue and resorbing the underside of the bone immediately adjacent to the necrotic periodontal ligament area (16, 17).

The level of tissue response has been correlated with the magnitude of the applied force. Schwarz (12) concluded that forces delivered as part of orthodontic treatment should not exceed the capillary-bed blood pressure (20-25 g/cm^2 of root surface) as the compression can cause tissue necrosis. Greater forces would result in physical contact between the root surface and bone, resulting in undermining resorption or hyalinization. To reduce the amount of degeneration, the amount of force per unit area should be minimised (18).

Thus, orthodontic forces are considered, according to the “pressure-tension” theory, to generate a compression and tension zone, and these were traditionally believed to cause resorption and apposition, respectively. The loading of teeth and the subsequent bony changes allow for tooth movement. This, however, conflicted with the concepts of the bone biologists, who considered that loading resulted in bone formation.

Bone-bending theory

The role of alveolar bone bending in orthodontic tooth movement was first proposed by Farrer in 1888 and further investigated in the rat model (13) and in humans (19). When orthodontic pressure is delivered to a tooth, the forces are transmitted to all tissues within the region of force application. This results in bending of the tooth, bone, and solid structures of the periodontal ligament. As bone is more elastic than tooth or periodontal ligament components, it bends more readily and is associated with active biological processes. The bone is held in a deformed position, activating biological processes and accelerating bone turnover. This results in remodelling of the bone, changing its shape and internal organisation to accommodate the orthodontic forces acting upon it.

Bioelectric signalling theory

In response to applied mechanical forces, it has been proposed that there is generation of electric potentials within the stressed tissues (20-22). These currents may charge macromolecules that interact at specific sites or mobilise ions across cell membranes (23). This 'piezoelectric' phenomenon was observed to occur in mechanically stressed alveolar bone *in vitro* and *in vivo* (24, 25). Zengo and coworkers demonstrated that the concave side of the orthodontically treated bone is electronegative and favours osteoblastic activity, whereas the convex surfaces are associated with positivity or electrical neutrality and elevated osteoclastic activity (24, 25).

Davidovitch and coworkers (1, 26) administered exogenous electrical currents in conjunction with orthodontic forces, resulting in increased rate of tooth movement and increased degree of tissue remodelling. It was concluded that bioelectric responses, including piezoelectricity and streaming potentials, caused by bone bending due to orthodontic force application, may function as pivotal cellular first messengers. Of concern, however, was the potential for tissue damage near the anode, should the amount of electric current be too great. This could potentially have had direct inflammatory effects as a result of thermal damage to the tissues. Davidovitch et al. considered that a physical relationship existed between mechanical and electrical perturbation of bone and that the bending of bone (collagen, hydroxyapatite, or bone cell surfaces) caused stress-generated electrical effects. Other sources of stress-generated potentials have also been proposed (27, 28).

Phases of tooth movement

It has been proposed that, based upon rates of tooth movement over time, there are three distinct phases of tooth movement: an initial phase, a lag phase, and a post-lag phase, in which the majority of the total orthodontic tooth movement occurs (29). Studies using beagle dogs modified this hypothesis, suggesting that tooth movement takes place in four phases (30-32).

- Phase 1: 1-2 days duration, representing the immediate, rapid initial movement of the tooth inside its bony socket following the application of force to the tooth. The tooth is displaced within the periodontal ligament space.
- Phase 2: 20-30 days duration, where tooth movement ceases and necrotic tissue is formed through the hyalinization of the periodontal ligament in areas of compression.
- Phase 3: The necrotic tissue is removed, resulting in accelerated tooth movement (31).
- Phase 4: Continuation of accelerated tooth movement.

In the initial phase of tooth movement, cellular and molecular reactions commence immediately after force application. The force-induced tissue strain results in pro-cellular matrix reorganisation and the synthesis and release of various neurotransmitters, cytokines, growth factors, and metabolites that act as secondary messengers on signal transduction pathways, for example, arachidonic acid and prostaglandins.

In the lag phase, areas of compression are characterised by the distorted appearance of the periodontal ligament fibres and recruitment of phagocytic cells to remove necrotic tissue from compressed periodontal ligament (PDL) sites and adjacent alveolar bone. The phagocytic cells remove the hyalinized tissue that develops as a result of the disruption in blood flow to the region. Removal of the necrotic tissue, combined with bone resorption, results in resumption of tooth movement. In areas of PDL tension, osteoblasts are enlarged and start producing new bone matrix. There is an increase in the number of new osteoblasts as a result of recruitment of fibroblast-like cells around PDL capillaries, and these cells migrate toward the alveolar bone surface. Simultaneously, PDL fibroblasts begin to proliferate and remodel their surrounding matrix in the tension zone.

The acceleration and linear phases of orthodontic tooth movement commence approximately 40 days after the initial application of force. The presence of hyalinization zones in compression areas suggests that the development and removal of necrotic tissue is a dynamic and continuous process during tooth movement. Additionally, in the compression zone, the bone surfaces are irregular, as is the orientation of the collagen fibres (32).

Biology of orthodontic tooth movement

Orthodontic tooth movement is a mechanically mediated inflammatory process. The application of orthodontic forces, which can be considered to be static and therapeutic, results in a response within the alveolar process. The mechanical distortion of cell membranes results in activation of phospholipase A_2, making arachidonic acid available for the action of cyclooxygenase and lipoxygenase enzymes, such as prostaglandin E and prostacyclin (PGI_2) (33). The prostaglandins bind to cell membrane receptors, resulting in the stimulation of secondary messengers, which leads to a cell response. This control process affects cells along the bone surface, as well as osteocytes that are situated in a rigid matrix. Osteocytes are mechanosensory cells that are able to translate mechanical strain into biochemical signals that regulate bone modelling and remodelling. These cells are ideally positioned to detect changes in mechanical stresses and to relay signals to surface lining osteoblasts, which progress to bone formation and bone resorption. Prostaglandin EP2 receptors are involved because signals are transmitted across gap junctions between osteocyte processes (34). This activates the cAMP-protein kinase pathway, which has been implicated in tooth movement (35). Other secondary messengers, such as inositol phosphate and intracellular calcium, are also involved and their activation will evoke a nuclear response, which will either result in production of factors involved in osteoclast recruitment and activation, or bone-forming growth factors. Thus, the stimulation of osteocytes supports osteoclast formation and activation.

Osteoblasts have receptors for many of the hormones and growth factors that stimulate bone modelling and remodelling. In contrast, the osteoclast is comparatively insensitive to these signals. Osteoclasts are more responsive to inhibitory signals, such as calcitonin and prostaglandin, which inhibit them from resorbing calcified matrix. Osteoblasts are responsible for the recruitment and activation of osteoclasts when they are stimulated by various hormones. Osteoblasts activate osteoclasts through the OPG/RANK/RANKL regulatory system (36).

Orthodontic tooth movement involves two interrelated processes: deflection or bending of alveolar bone and remodelling of periodontal tissues (37), which is a factor that distinguishes the remodelling process from typical bone responses. The role of the PDL, which is considered to be an extension of the periosteum, is essential because the PDL is intimately connected to the bundle bone of the alveolus and the cementum of the root. It could be considered that the tension within the fibres of the PDL are transmitted to the adjacent structures, but it has been found that the PDL behaves as a viscoelastic gel that flows and bounces and hence forces are not transferred (38). Additionally, when the PDL is disrupted, orthodontic tooth movement still occurs (39). Another differing factor is that during orthodontics, the forces applied to the teeth are intermittent, rather than constant. This is due to the role of the occlusion, which causes a ‘jiggling’ effect as teeth come into occlusal contact.

During tooth movement, should the orthodontic force be too great, the pressure in the PDL becomes too high, resulting in hyalinization and indirect resorption of bone at a distance from the PDL. The indirect resorption is considered to be an ‘undermining resorption’ starting from the adjacent bone marrow (40, 41) and occurs in the absence of formative activity on the tension side of the tooth. This is because only a minor displacement of the tooth occurs. The periodontal ligament in the region is compressed and hyalinization due to excessive compression and local tissue necrosis may occur (42). This hyalinised or necrotic tissue is removed by phagocytic cells (43) when the undermining resorption reaches the PDL. At this point, the tooth begins its displacement and loosens due to the widened PDL (40). Once tooth movement commences, bone apposition occurs at the tension side, followed by either renewed hyalinization or a continuation of the tooth movement through direct resorption of the alveolar wall. With no compensatory apposition of bone, there is a net loss of bone and the tooth may be moved outside the alveolar process without bone coverage.

The determining factor for the bone response to tooth movement is the stress/strain distribution in the PDL, which is modified by the magnitude of force, bone area, and type of tooth movement (40). The amount of force and the stress/strain distribution determines the biological reaction and, using Frost’s mechanostat theory (44), it was considered that the direct resorption of bone could be due to lowering of the normal strain from the functioning periodontal ligament, resulting in increased remodelling space. Physiological loads would balance the resorption and formation, producing new lamellar bone. Excessive strains would result in a negative balance as repair would not be able to keep up with the occurrence of microfractures (45) and may produce woven bone (26, 46). This would result from excessive loads that would be considered to be traumatic.

Frost (44) described the presence of woven bone as a response seen when the stimulus exceeded a certain value, below which lamellar bone was formed. He also determined that 1500-3000 μm is a typical minimum effective strain for lamellar bone to start modelling, and that if the strain is below 100-300 μm, remodelling is activated as a result of inactivity (47).

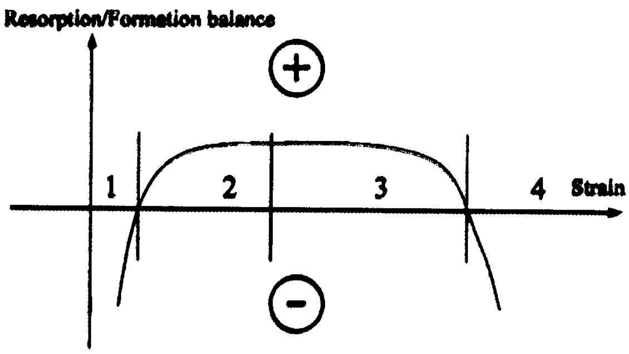

Figure 1: Graphical illustration of bone biological reaction to variation in strain values. In the case of low strain values (1), remodelling is turned on and a negative balance will be the result. With higher strain values (2), modelling is turned on and formation of lamellar bone occurs. Further increase in strain (3) results in the formation of woven bone. Severe overloading (4) results in negative balance due to the repair process related to the microfractures occurring at the strain level. From Melsen (48).

Melsen (48) in 2001 proposed a new paradigm for tissue reactions to orthodontic tooth movement. This aimed at overcoming the conflicting beliefs between groups: orthodontists generated resorption by applying pressure whilst orthopaedic surgeons caused bone apposition through loading. The main argument for consistency between the two conflicting views was based upon a study by Epker and Frost (49), which showed that the stretching of the PDL resulted in a 'bending of the bone' in the tension zone and hence that the apposition of the alveolar wall could be considered to be a reaction to bending. In the monkey model, Melsen found that strain values in the direction of displacement (or the ‘compression’ zone) were below the minimum effective strain (48). This was due to compression of the PDL and thus would cause underload remodelling, accounting for the direct resorption on the ‘compression’ side. On the ‘tension’ side, the PDL fibres were stretched, generating a strain level corresponding to modelling, thus causing new bone formation. Additionally, woven bone was seen ahead of the alveolus in the direction of the displacement.

Melsen also found that the PDL fibres were stretched and that formation activity was found along the major part of the alveolus, leading to the delivery of strain values corresponding to modelling. As it was an intrusive movement, the apical fibres were not stretched and hence the apical bone resorption was thus interpreted as remodelling.

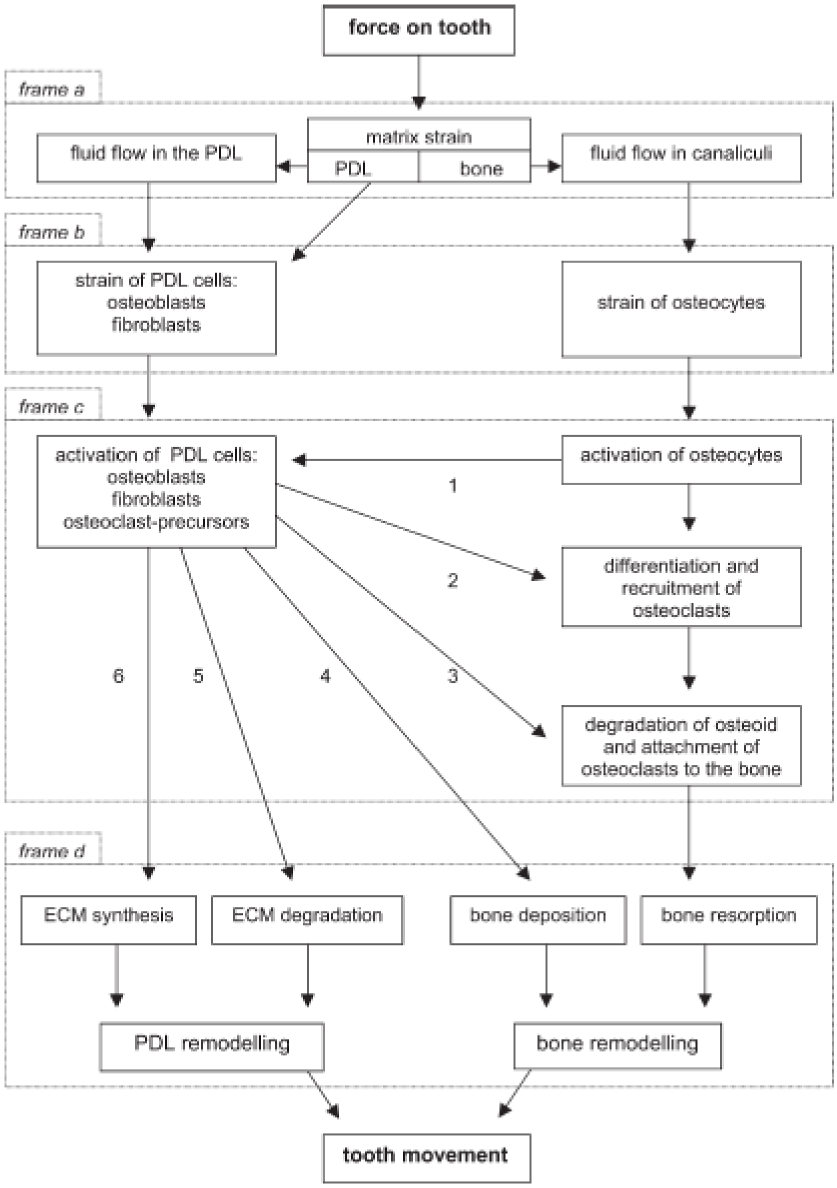

Figure 2: A theoretical model of tooth movement. The model describes four different stages in the induction of tooth movement. Frame (a) represents matrix strain and fluid flow, (b) cell strain, (c) cell activation and differentiation, and (d) remodelling of the periodontal ligament (PDL) and bone. From Henneman et al. (50)

Henneman et al. (50) presented a theoretical model that involves four stages in the induction of tooth movement (Figure 2). Immediately after the application of a force, the tooth is able to shift a small distance within its socket. This results in a negative strain (compressive deformation) within the PDL on the future resorption side of the root (51), relaxing the collagen fibres. Conversely, there is positive strain (tensional deformation) within the PDL on the future apposition side of the root (51), with stretching of collagen fibres connecting the tooth to bone (52). This is depicted in Figure 3.

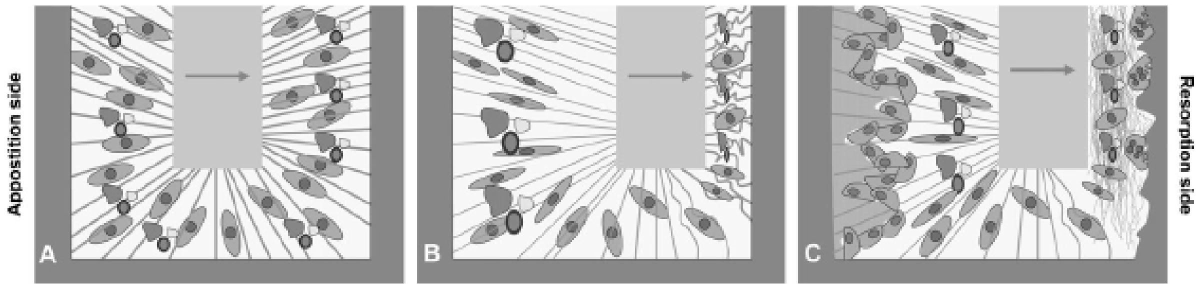

Figure 3: Schematic drawing of a tooth, the periodontal ligament with cells, and alveolar bone. (a) An external force is applied (arrow). (b) At the apposition side, fibres are stretched. Compression of fibres takes place at the resorption side. (c) After prolonged application of force, bone formation by osteoblasts can be found at the apposition side and osteoclasts resorb the bone at the resorption side. From Henneman et al (50).

The application of an external force on the tooth causes fluid flow in the PDL and in the canaliculi, leading to shear stress on osteocytes (53). This can result in apoptosis of osteocytes, subsequently leading to the attraction of osteoclasts (54). Additionally, microcracks occur in bone as a result of material fatigue, which may provide further stimulus to apoptosis and attraction (55). Verna et al. (56) found that there were increased numbers of microcracks present at the resorption side during the initial phase of orthodontic tooth movement in pigs. These cracks reflect areas of initial damage of the bone that will be remodelled at later stages.

Following the stages of cell deformation and activation, osteocytes produce specific cytokines (such as prostaglandins and TNF-alpha) that activate osteoclast precursors in the PDL side (57), while nitric oxide inhibits osteoclast activity at the apposition side (58). The remodelling process is induced through the complex regulatory network of fibroblasts, osteoblasts, and osteocytes, resulting in tooth movement.

At the resorption face, osteoclast precursors migrate to the bone surface and differentiate into osteoclasts. Activated osteoclasts dissolve the inorganic and organic matrix, creating space for tooth movement and removing the attachment of the principal fibres of the PDL to bone. At the apposition side, the PDL fibres are stretched, stimulating osteoblasts to produce new extracellular matrix and to mineralise the osteoid. The new bone is thickened, trapping osteocytes and PDL, forming new Sharpey's fibres. The cycle continues until the removal of the orthodontic force.

Thus, the rate of tooth movement is dependent upon the rate of bone turnover and, as normal bone remodelling would proceed at too slow a rate to allow efficient tooth movement, a phenomenon to increase the rate of remodelling is an essential factor in orthodontics. Its absence would make it impossible to perform tooth movement, significantly increasing overall treatment times beyond the lifespan of the patient.

Regional Acceleratory Phenomenon

The regional acceleratory phenomenon (RAP) was first described by Frost (6) and is defined as 'a complex reaction of mammalian tissues to diverse noxious stimuli'. RAP is a phenomenon that affects the skeletal and soft tissues in an anatomical area. Both the stimulated area and surrounding tissues are affected (59). RAP is characterised by an acceleration of ongoing normal vital processes and may be considered to be a protective mechanism that evolved to potentiate tissue healing and fortify local tissue immune reactions. The response in the bone may be considered to be exaggerated or intensified. This is recognisable when the RAP is hindered, because deficient healing, reduced resistance to infection, and mechanical abuse may occur.

Causes

RAP is initiated when a regional noxious stimulus of sufficient magnitude affects the tissues. Frost (6) observed that the size of the affected region and the intensity of its response varied directly with the magnitude of the stimulus, though there was individual variation in the degree of the response. The noxious stimulus can greatly vary in nature and can include any perturbation of bone, including traumatic injuries, fractures (60), osseous surgery (61-64), vascular surgery, crushing injuries, thermal trauma, infections (65), and most non-infectious, inflammatory joint processes, including rheumatoid arthritis (47). RAP can also occur by extracting a tooth (66), raising a periodontal flap (2), or placing a dental implant (67).

Nature

Once RAP has been evoked, normal regional hard- and soft-tissue vital processes accelerate above normal response levels. Collectively, these accelerated processes represent the RAP and they include: growth of connective tissue structures (60, 68), remodelling of connective tissues (13), skin epithelialisation, soft tissue and bone healing, perfusion (69), and cellular turnover and metabolism (47). RAP does not seem to provide new processes but increases the rapidity of healing through all the post-fracture stages, including granulation, modelling, and remodelling (70). This results in healing occurring two to ten times more rapidly than otherwise, which means that additional remodelling cycles of resorption followed by formation are activated.

The affected region, as a result of the acceleration of these processes, exhibits inflammatory changes, including erythema and oedema, subsequently increasing temperature and bone turnover. Hot regions found in acute and chronic osseous conditions, including osteomyelitis, healing fractures, joint inflammation, and bone metastases, can be attributed to the uptake of bone-seeking isotopic agents. Frost (6) suggested that the appearance of the cardinal signs of inflammation represented an early recognised manifestation of a stereotyped, more general phenomenon.

In a situation involving the fracture of bone, as was being examined by Frost (6) and Lee (71), the RAP response is divided into phases, with an initial phase of maximally stimulated bone formation in which woven or fibrous bone is produced to span a cortical gap (72). This new bone is eventually remodelled into lamellar bone. This process is followed by a period of predominant resorption, in which medullary bone disappears and the number of osteoblasts decreases. This decreased regional bone density due to increased modelling space may also lead to regional tissue plasticity (6). The increased intracortical bone remodelling produces tunnelling within the cortex that can be seen on clinical radiographs. It is postulated that osteoclast and osteoblast cell populations shift in number, resulting in an osteopenic effect (59, 73).

As well as variation due to different causes of RAP, the response will depend upon the anatomy, competence and autonomic innervations of the regional blood supply, regional sensory innervations, and mechanical loading, as well as local biochemical and biological factors known to be associated with injury, repair, metastasis, and inflammation (6). Frost proposed several mechanisms for RAP, such as a decrease in osteoblast cell number, cell proliferation responses, neovascularization, and local and systemic mediators (70, 74).

Anatomical distribution

The RAP involves the anatomical region where its stimulus arose, such the periodontium surrounding a tooth during orthodontic tooth movement. The distribution of the RAP reflects the regional vascular anatomy and innervations, allowing transition of the phenomenon from involved to uninvolved regions. This is a gradual change, rather than an immediate or rapid shift. Additionally, it has been observed that with severe stimuli, RAP can occur in contralateral regions of the body (6).

Duration

Frost (75) estimated the total duration required for the remodelling – activation, resorption, and formation (ARF) – to be 12 weeks. The duration of the RAP depends upon the severity of the stimuli, although in healthy humans, a single traumatic stimulus, such as a gunshot wound, will result in clinical evidence of RAP of approximately four months duration in bone (6). RAP begins within a few days of the fracture, typically peaks at one to two months, and may take six to more than 24 months to subside (70). The duration in soft tissues is shorter. With more severe trauma, such as acute paralysis or severe thermal burn, the RAP can last from six months to over two years. Prolonged stimulation, such as that resulting from rheumatoid arthritis, osteomyelitis, Paget's disease, or osteoid osteoma, can produce a persistent RAP without limit to its duration.

Clinical application of Regional Acceleratory Phenomenon

In normal conditions, less than 5% of the adult human tibial compacta is remodelled annually (76). Should a fracture occur, and if no other phenomenon modifies the healing response, less than 5% of the tibial fracture interface would bridge within the first year, and complete bridging would require over 20 years of healing time. However, due to trauma as a result of the fracture and also the reparative surgery, the local bone turnover is accelerated ten- to fifty-fold above normal for more than a year. This allows the union to occur within six months and is accompanied by a concurrent acceleration in the healing process of the soft tissues. Conversely, should the RAP fail to promote fracture callus formation sufficiently, a fracture non-union will result (less than 3% of all fractures) (74). Thus, it is suggested that normal fracture healing may routinely require an accompanying RAP and its absence may result in a delayed union.

Additionally, according to 'Wolff's law', living bone can modify its internal architecture in response to an alteration in applied mechanical loads. This ensures that the bone architecture is optimally prepared mechanically to support altered loads (77, 78). Extrapolation of this concept suggests that mechanical usage of a bone can influence its architecture and that the reported bone reactions are typical RAP manifestations (62).

The rate of remodelling, when elevated as a result of the regional acceleratory phenomenon, has been shown to increase the rapidity of tooth movement (79-81). Verna et al. (82) investigated the influence of bone metabolism on the rate and the type of orthodontic tooth movement in the rat model. In comparing groups with high, low, and control rates of bone turnover, it was found that the bone turnover significantly affected the rate of tooth movement. As a result, one area of significant interest is increasing the rate of bone turnover through the utilisation of RAP associated with orthognathic surgery. The advent of rigid fixation allows orthodontists to take advantage of the surgically induced RAP to achieve extensive orthodontic tooth movement postoperatively (83).

Melsen (52) investigated the relationship between the strain levels and biological tissue reaction in monkeys by using closed coil springs to achieve tooth translation. By comparing this with unloaded control teeth, they observed a relative extension of resorption from 3-5% in the control group to 7-13% of the total cancellous surfaces surrounding the loaded teeth. There was also an increase in extension of appositional surfaces from 15-20% in the controls to 35-49% around the loaded teeth. Relative to control teeth, the density in the direction of tooth movement was increased by a factor of 2 to 3. The alveolar wall in the direction of the tooth movement was completely resorbed, while woven bone formation was seen in the alveolar bone ahead of the direction of tooth movement. The extension of affected region and the intensity of the response varied directly with the magnitude and nature of the stimulus.

Verna et al. (84) used a rat model to examine the regional effects of orthodontic tooth movement. An orthodontic force was applied to mesialise the maxillary first molar, but bony changes were visible histologically around all the teeth in the region. Verna considered this force to be perceived as a noxious stimulus against which the surrounding bone developed a defensive mechanism. It was concluded that not only the alveolar bone surrounding the alveolar socket of the tooth was affected by the mechanical perturbation, but also the bone that surrounded the adjacent teeth. Verna also argued that because there was new bone formation on the periosteal side at an early stage (85), followed by further bone formation at a later stage, that the first periosteal response is non-specific whilst the later response is the result of a regional acceleratory phenomenon.

Corticotomy

A corticotomy is defined as a surgical procedure in which the cortical bone is cut, perforated, or mechanically altered without involvement of the medullary bone. In laboratory studies, when a surgical incision was made into the head of the tibia in rabbits, new bone, including trabecular bone, formed around the incision area as a result of increased bone turnover (59, 86). Furthermore, orthodontists have long noted increased rates of tooth movement following orthognathic surgical procedures, though this effect is usually attributed to a postoperative acceleration of bone remodelling. A consequence of this observation is that maxillary corticotomy is now a routine procedure for surgically assisted rapid palatal expansion (87, 88). However, alveolar corticotomy to enhance the rate of tooth movement has developed more slowly, largely because of concerns about periodontal outcomes.

The most widely known benefit of the modern corticotomy procedure is faster tooth movement, with some authors claiming it to be three to four times quicker than traditional orthodontic movement (54). Shorter treatment time may provide greater motivation for patients. More importantly, these procedures can modify the dentoalveolar complex so that the teeth, alveolar bone, and skeletal components can be appropriately addressed for maximising ideal functional and aesthetic relationships. In addition, these techniques could reduce root resorption and provide a more stable result than traditional cell-mediated tooth movement alone (5). The shortened treatment time reduces the risk of periodontal inflammation, dental caries, and decalcification (89, 90). The correction of numerous interdisciplinary dental-facial problems will result in arches that are perceived to be more aesthetically appealing in modern society.

It was believed that the rapid tooth movement after corticotomy surgery was due to the movement of small outlined blocks of bone with the teeth as handles (91). The resistance of the cortical layer of bone was presumably eliminated with the circumscribing corticotomy cuts. The only resistance to the tooth movement would thus be provided by the less dense medullary bone. It was thought that this could overcome the slow PDL-mediated process of traditional orthodontics because the tooth–PDL complex was being moved with the block of bone and not through the bone. Corticotomy was often a highly morbid, hospital-based procedure requiring surgical cuts to be made entirely through the buccal and lingual alveolar process periapically. The old 19th and 20th century version of the corticotomy procedure risked the devitalisation of teeth as well as alveolar necrosis. Moreover, some surgeons made such deep bony cuts interproximally that the orthodontist considered it to be regional orthognathic surgery. Since this unrefined technique lacked an evidence base and had a high morbidity, it justifiably enjoyed rather little popular support (92).

Iino et al. (93) found that the insult of circumscribing corticotomy cuts alone does not elicit an osseous response that is sustainable enough to permit tooth movement through a large thickness of bone in the mesio-distal orientation of the alveolus. Thus, they suggested that bone thinning be accomplished with an ostectomy through the entire thickness of the alveolus to include the labial and lingual cortical plates and interspersed medullary bone.

In 2001, Wilcko et al. (94) reported a revised corticotomy-facilitated technique that included periodontal alveolar augmentation, called accelerated osteogenic orthodontics (AOO) or periodontally AOO technique (PAOO). This technique demonstrated acceleration of treatment in two cases, reducing the usual overall treatment time by two-thirds.

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(a) *(b)* *(c)*

Figure 4: Accelerated osteogenic orthodontics (AOO) technique. (a) Inter- and supra-dental corticotomy diagram. (b) AOO corticotomy. (c) AOO grafting. From Ferguson et al. (95).

AOO involves a combination of “bone activation” (selective alveolar decortication, ostectomies, and bone thinning with no osseous mobilisation), alveolar augmentation using particulate bone grafting material, and orthodontic treatment. Facial and lingual surgical flaps are elevated and the cortical bone adjacent to the teeth to be moved is scored with a surgical bur penetrating barely into medullary bone. AOO technique employs a bone graft over the bleeding cortical bed, but the graft is not essential to induce alveolar osteopenia. The principal objective of the AOO surgery is the creation of a relatively thin layer of bone (approximately 1.5 mm) over the root prominence in the direction of the intended tooth movement. The design of the corticotomy cuts and perforations is not important but only needs to perforate the cortical layer of bone and extend into the superficial aspect of the medullary bone.

The corticotomy surgery acts as a noxious insult to the area, causing the induction of the alveolar structures into a more pliable condition favouring rapid tooth movement. There is a substantial increase in alveolar demineralisation resulting in a transient and reversible condition (osteopenia). Calcium is released from alveolar bone, resulting in a decrease in bone mass (mineral content or density) but no change in bone volume. Longitudinal tunnelling takes place in cortical bone, while both surface resorption and osteocytic osteolysis converts as much as 50% of local trabecular bone to osteoid in six weeks. The osteopenia enables rapid orthodontic tooth movement because teeth are supported by and moved through trabecular bone. Bogoch (59) found a five-fold increase in bone turnover in a long bone adjacent to a corticotomy surgery site. The rapid tooth movement associated with corticotomy-facilitated orthodontics is more likely the result of a demineralisation/remineralisation process consistent with the initial phase of the regional acceleratory phenomenon, namely an increase in cortical bone porosity and a dramatic increase of trabecular bone surface turnover due to increased osteoclastic activity. As long as tooth movement continues, the RAP is prolonged. When RAP dissipates, the osteopenia also disappears. When orthodontic tooth movement is completed and retainers are delivered, an environment is created that fosters alveolar remineralisation.

Figure 5: Osteocytic osteolysis within trabecular lamellar bone showing cutting cone and secondary osteon formation. Osteoclasis is followed by bone apposition and osteoid formation. Mineralisation begins between 20 to 55 days after osteoid formation. From Ferguson et al. (95).

Figure 6: Diagrammatic comparison of steady state vs RAP-induced bone resorption with hypertrophied osteocytes and increased number of osteoclasts. From Ferguson et al. (95).

Figure 7: Diagrammatic comparison of steady state vs RAP-induced bone formation with high amount of demineralised bone (osteoid). From Ferguson et al. (95).

Recently, Binderman and co-workers (96) suggested that the major stimulus for the alveolar bone remodelling that enabled periodontally accelerated osteogenic orthodontics was not RAP. Instead, the stimulation was attributed to the detachment of the bulk of dentogingival and interdental fibres from the coronal part of the root surfaces, which the authors considered to be sufficient to stimulate alveolar bone resorption and to lead to widening of the periodontal ligament space. This would allow accelerated osteogenic orthodontic movement of teeth. Additionally, the fiberotomy would transiently disrupt the positional physical memory of the dentition, allowing accelerated tooth movement and reducing relapse. The basis of the argument by Binderman and co-workers is that the episode of osteoclastic alveolar bone and soft tissue remodelling is attained through the elevation of a full-thickness mucoperiosteal flap alone, without surgical wounding of the cortical bone (2, 97-99).

Additionally, in the rat model, alveolar bone resorption has been shown to occur when full-thickness flap surgery is performed by a coronal approach (sulcular incision), whereas an apical surgical approach, without disruption of the gingival attachment to the root surface, does not result in significant alveolar bone remodelling (99). Binderman and co-workers (95) concluded that mucoperiosteal flap surgery could be separated into two procedures: (1) surgical detachment of dentogingival and interdental fibres, which produces a strong signal for osteoclastic bone resorption on the inner aspect of the PDL facing the tooth, and (2) separation of mucoperiosteum from bone and corticotomy, which produces a burst of regional bone remodelling that is consistent with RAP. It should also be noted, however, that at the control sites in the experiment, where only surgical incisions were performed without elevation of the periosteum, no alveolar bone resorption was observed. Additionally, although less alveolar bone height loss was found at apical approach sites, which was expected because the gingival margin was not involved, evidence of bone remodelling was present histologically on the surface of the alveolar bone. Thus, surgical incisions, which are comparable to a fiberotomy procedure, did not result in alveolar bone resorption and elevation of the mucoperiosteum (coronally and apically) results in alveolar bone changes, though possibly only on the surface of the alveolar bone.

Case reports

There are numerous case reports involving pre-orthodontic corticotomy and acceleration of orthodontic tooth movement, including a publication by Köle in 1959, which was the first to describe modern-day corticotomy-facilitated orthodontics (100). Köle believed that the surgical preparation of the alveolus would permit rapid tooth movement, suggesting that it was the continuity and thickness of the denser layer of cortical bone that offered the most resistance to tooth movement. He theorised that by disrupting the continuity of this cortical layer of bone, he was actually creating and moving segments of bone in which the teeth were embedded. These outlined blocks of bone could be moved rapidly and somewhat independently of each other because they were connected only by less dense medullary bone, which would act as the nutritive pedicle, maintaining the vitality of the periodontium.

From Köle's work arose the term '*bony block*' to describe the suspected mode of movement after corticotomy surgery (100). Köle used a combined interradicular corticotomy and supra-apical osteotomy technique for rapid tooth movement. Blocks of bone were outlined using vertical interradicular corticotomy cuts both facially and lingually, which were then joined 10 mm supra-apically with an osteotomy cut through the entire thickness of the alveolus. It was assumed that the surgically outlined blocks of bone retained their structural integrity during healing. By use of relatively gross movements accomplished with very heavy orthodontic forces using removable appliances fitted with adjustable screws, Köle reported that the major active tooth movements were accomplished in 6 to 12 weeks. Soon after the Kolë articles were published, many authors described orthognathic surgical techniques for correcting overall maxillary and mandibular skeletal discrepancies. The corticotomy procedures described by Kolë never became popular, probably as a result of the limited orthodontic appliances and techniques available to support them, as well as advancements in orthognathic surgery.

Anholm et al. (91) in 1986 recognised that corticotomy-facilitated orthodontic treatment had the potential to decrease treatment time greatly. Anholm described the corticotomy procedure as a surgical technique in which a fissure is made through the cortical plate of bone that surrounds a tooth so that the tooth is in a block of bone that is connected to other teeth and structures only through the medullary bone. The tooth is the handle by which this block of bone is moved through the less dense medullary bone. He also suggested that ankylosed teeth, which have lost their periodontal membrane, could be moved into their optimum position with this method. In Anholm's case report, corticotomy was performed on the maxilla facially and lingually from the first molar to the contralateral first molar and, in the mandible, from cuspid to contralateral cuspid in a case with a Class II dental relationship and severe constriction of the arches. The case was treated in 11 months with fortnightly adjustments. It was recognised at the time that there were still unanswered questions about corticotomy-facilitated orthodontics, particularly in relation to bone movement histologically.

Suya built upon the supra-apical horizontal osteotomy used by Köle. In these publications, the osteotomy cut was replaced with labial and lingual corticotomy cuts. It was reported that he had treated 395 patients by corticotomy-facilitated orthodontics with good results (101).

Gantes and co-workers (102) in 1990 reported on corticotomy-facilitated orthodontics in five adult patients in which circumscribing corticotomy cuts were made labially and lingually between the roots. The upper first bicuspids were removed and the bone over the extraction sockets was removed buccally and lingually. The mean treatment time for these patients was 14.8 months, with the distalisation of the canines mostly completed in seven months. The mean treatment time for a comparable orthodontic control group was 28.3 months. However, Gantes and co-workers did not thin the interseptal bone on the distal of the canine to be distalised, which could have further shortened the overall treatment time.

Liou (3) in 1998 showed rapid tooth movement following surgery as a consequence of changes in the physiology and/or composition of alveolar bone. He used a technique called “dental distraction” in which the mesial aspect of the socket of an extracted first premolar tooth was directly modified (surgically undermined) to allow “distal distraction” of the adjacent cuspid. The alveolus, the PDL, or both, were distracted into a new configuration followed by reorganisation. Liou claimed that there were no adverse effects to the periodontal support and that the PDL re-established integrity after a mean cuspid retraction of 6.5 mm in three weeks.

Owen (103) treated his own mild anterior crowding with corticotomies in the anterior mandibular region and Invisalign™ treatment, which was accelerated to completion in 8 weeks through changing of the aligners every 3 days.

Nowrazi (104) reported the use of an autogenous bone graft in conjunction with corticotomies to treat an adult with a Class II division 2 crowded occlusion. Total active orthodontic treatment was completed eight months after corticotomy surgery.

In 2006, Germec et al. (58) published a case report of lower incisor retraction in a 22-year-old patient with protrusive profile, severe anterior crowding, an anterior crossbite, and Class III dental relationship using a “modified” corticotomy technique. Vertical cuts were placed 2 mm into bone with a 0.5 mm round bur, followed by a chisel to reach the lingual cortical bone from the labial side. The lingual, vertical, and subapical horizontal cuts were eliminated. The study found that corticotomy-facilitated orthodontics dramatically reduced the treatment time without any adverse effects on the periodontium and the vitality of the teeth. The main advantages of this “modified” corticotomy technique were the elimination of the lingual cuts and flap, the reduction of surgery time, and reduced discomfort to the patient.

Placement of titanium miniplates and orthodontic treatment combined with corticotomy were performed on an adult patient with Angle Class I malocclusion with flaring of the maxillary and mandibular incisors. The total treatment time was one year and it was concluded that corticotomy-facilitated orthodontic treatment with titanium miniplates might shorten an orthodontic treatment period without any anchorage loss or adverse effects (105). The combination of corticotomies and orthodontic treatment has also been reported in the treatment of an anterior open bite with flared and spaced mandibular incisors with a total treatment time of five months (106).

Hwang and Lee (107) in 2001 showed two case studies of intrusion of over-erupted molars using corticotomy. Cuts were made on both the buccal and lingual sides and the 'block of bone' was retained only through the medullary bone. Repelling rare earth magnets were used to apply the orthodontic force immediately after the corticotomy. A heavier force of more than 90 g was applied on the molar but the study found no adverse effects, such as root resorption or periodontal damage. The treatment was completed in one month for the upper molar and three months for the lower molar. Additionally, the authors suggested that it was necessary to apply orthodontic forces immediately after the corticotomy to achieve the desired tooth movement. Otherwise, the procedure would lose effectiveness as the bone healed.

Figure 8: Intrusion of a lower molar: (A) U-shaped shadow of corticotomy; (B) after 3 months of intrusion; (C) 4 years post-retention. From Hwang and Lee (107).

Similarly, other case reports have been published upon the use of corticotomies to intrude supraerupted molars with accelerated results (108, 109).

Spena and co-workers (110) used segmental corticotomies to facilitate molar distalisation into a Class I relationship in 8 weeks. Hassan et al. (111) reported upon two case studies using corticotomy-assisted expansion (CAE), which was considered to be an effective treatment modality for unilateral crossbites and bilateral crossbites with different side severity in adults. This report was the first to describe the use of CAE to treat mild to moderate maxillary transverse deficiency in adults with greater stability and without compromising periodontal health. Decreased cortical resistance, increased bone remodelling, and bone augmentation seemed to allow safer and stable expansion in skeletally mature patients where slow palatal expansion is ineffective, dangerous and unstable.

Figure 9: Surgical procedure of CAE. (A) & (B) buccal and palatal incisions are made. (C) & (D) full thickness flap is reflected. (E) selective alveolar decortications lines and points are made. (F) & (G) bone graft is placed. (H) & (I) flap is sutured back. From Hassan et al. (111).

Figure 10: Initial (top) and final (bottom) intraoral composite photographs of case 1. From Hassan et al. (111).

Figure 11: Initial (top) and final (bottom) intraoral composite photographs of case 2. From Hassan et al. (111).

Wilcko demonstrated AOO using two case reports (94). Case A involved a non-extraction treatment of severe upper arch constriction in the anterior/premolar areas, with bilateral crossbites and severe upper and lower crowding. Appliances were activated every 2 weeks and treatment was completed in 6 months and 2 weeks. Pre-treatment and post-treatment cross-sectional analysis of the computed tomographic scan through the lower left central incisor showed an increase in the alveolar bone width of 2.4 mm at B-point and 3.5 mm lingually. The increase in the thickness of the alveolar housing was readily apparent at the one biopsy site on the facial of the upper left first bicuspid, where there was a thickness of 3 to 4 mm of new healthy bone post-treatment.

Case B involved unilateral space closing in an adult that was completed in 7 months. At 8 years after surgery, the lower right area was re-exposed and examined. At the time of the initial surgery, there was a bony dehiscence on the facial of the root of the lower right canine that extended almost to the apex of this tooth. This dehiscence at re-examination was completely filled with bone. A bone biopsy was removed from the facial of this tooth and the sample was found to be healthy lamellar bone.

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Figure 12: (A) After full-thickness flap reflection and before bone activation, there is a fenestration on the facial of the lower left canine that extends almost to the apex of the root. (B) Re-entry by a full-thickness flap 17 months after AOO surgery (10 months after de-bracketing); the bony fenestrations are filled in with new bone. (C) After removal of cores of bone from the facials of the lower left canine and lower left lateral incisor, where there had originally been no bone due to the facial fenestrations, there is now 2- to 3-mm thickness of bone. From Wilcko et al. (94).

In 2007, Kanno (112) presented a case report involving corticotomy and compression osteogenesis, identified as an osteoplasty technique based on the distraction osteogenesis phenomenon. Corticotomies were performed over 2 stages, with an initial corticotomy on the palatal surface of the upper first and second premolars with a mucoperiosteum incision on the alveolar ridge 3 mm above the apices of the teeth. Three weeks later, a corticotomy of the buccal surface was performed. Repositioning of corticotomised bone/teeth segments was achieved within a month, using elastics inducing gradual compressive segmental movement.

Lee (71) showed the difference between corticotomy- and osteotomy-assisted tooth movement using microtomography imaging in the rat model. RAP was observed in the alveolar bone of the corticotomy-treated animals, while distraction osteogenesis was observed in the animals that underwent osteotomy-assisted tooth movement. These different bone reactions can be exploited for tooth movement. The changes with distraction osteogenesis were attributed to fracture-like healing around the mobile osteotomised segment. The post-corticotomy healing may be a result of enhanced healing potential due to openings into the underlying marrow vascular spaces.

Sebaoun et al. (113) reported that, in a rat model, selective alveolar decortication resulted in a 3-fold increase in the catabolic and anabolic processes at 3 weeks after surgery. There were increased numbers of osteoclasts, increased rate of bone apposition, decreased calcified spongiosa, and greater periodontal ligament surface area around the roots of teeth. By 11 weeks after surgery, this had dissipated to a normal steady state. As tooth movement was not included in the experimental design, the dynamics of the periodontal change in response to the decortication injury was clearly shown. The study also revealed that increased bone turnover was localised to the area immediately adjacent to the injury. Selective alveolar decortication resulted in a transient osteopenia and increased tissue turnover, the degree of which was directly related to the intensity and proximity of the surgical insult.

Corticotomy can be a viable treatment option to provide more controlled differential expansion (as well as unilateral expansion) than conventional expansion since tooth movement is expected to be more enhanced at the corticotomised site than at the non-corticotomised site. In Case 1, corticotomy was performed on the buccal and palatal sides of the right segment as described by Wilcko and co-workers (54). Expansion commenced 10 days after the corticotomy using fixed orthodontic appliances and a heavy labial archwire. This was done without the use of slow expansion or surgically assisted expansion, which require bulky conventional palatal expanders, hence making this method ideal for adult patients who do not tolerate palatal expanders. Crossbite correction was achieved in 10 weeks.

Corticotomy was performed only on the crossbite side to overcome the unnecessary contralateral expansion and to encourage increased unilateral tissue turnover and accelerated tooth movement. Although expansion theoretically occurred faster on the crossbite side than on the normal side, some expansion was also observed on the normal side, mainly due to tipping. This relapsed quickly after removal of the expander.

In Case 2, a corticotomy was performed differentially: buccal and palatal on the right side and only buccal on the left side. Expansion started 10 days post-corticotomy and was performed using a quad-helix appliance. After 12 weeks, over-correction was achieved, the quad-helix removed, and upper and lower pre-adjusted appliances were used for aligning, levelling, arch coordination, and finishing.

Expansion on the corticotomised side was believed to be bodily in nature and more stable. The authors used the ruler of the American Board of Orthodontics grading system to show that the level of buccal and palatal cusps of molars and premolars were the same before and after treatment. This is in comparison to the conventional methods of expansion in skeletally mature patients in which expansion is expected to be tipping in nature. However, the authors did warn that CAE should be limited to moderate skeletal discrepancies and is not a replacement for surgically assisted rapid palatal expansion (SARPE) in severe forms of palatal constriction.

In 2009, Chung (114) described a ‘new’ type of corticotomy-assisted orthodontic treatment called “speedy orthodontics” for treating severe anterior protrusion in adults as an alternative to orthognathic surgery. Corticotomy alters the shape of the medullary bone and when the bone ossifies, it is prevented from returning to its original form. Speedy orthodontics describes a protocol that allows movement of dental segments over a shorter time by using a corticotomy and an orthopaedic force with temporary anchorage devices. A greater than normal orthodontic force was applied, with the aim of moving the block of bone that was circumscribed rather than moving teeth through the bone.

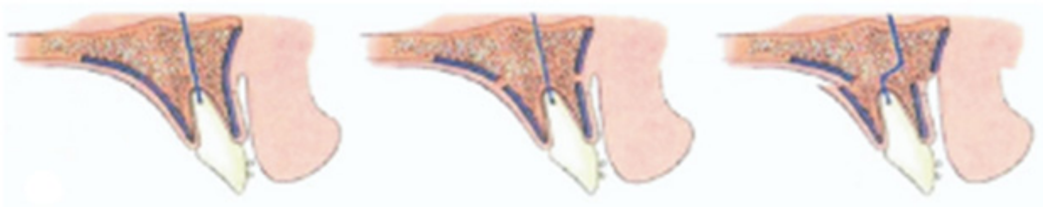

Figure 13: Schematic illustration of speedy orthodontics and sequence of anterior retraction after corticotomy. The medullary bone around the anterior teeth can be easily bent by retraction force if the cortical layer between the basal bone and the alveolar bone is removed. Adapted from Chung et al. (114).

Aboul-Ela and co-workers (115) evaluated miniscrew implant-supported maxillary canine retraction with and without corticotomy-facilitated orthodontics in 13 adult patients. The corticotomy procedure perforated the bone and was performed on the buccal side, leaving the lingual cortical plate intact. Miniscrews were placed buccally between the maxillary second premolars and the first molars. The authors showed that in maximum anchorage cases, using skeletal anchorage combined with corticotomy shortened the treatment time compared to the control (non-operated) side. The average daily rate of canine retraction was significantly higher with the corticotomy, being twice the rate of the control side during the first two months after the corticotomy surgery. This rate declined to only 1.6 times in the third month and 1.06 times by the end of fourth month. This result is consistent with the transient nature of the RAP. This study is in agreement with those of Wilcko et al., Iino et al., Ren et al. and Mostafa et al. who reported that tooth movement velocity on the corticotomy site was 2 to 3 times faster than on the control side (5, 93, 94, 116). However, this study has a few weaknesses. The study sample size was small (5 men and 8 women) and the contralateral arch was used as the control.

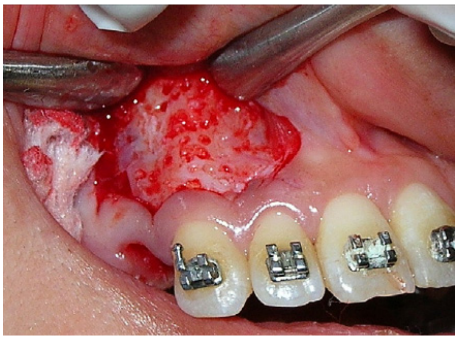

Figure 14: Flap design with cortical perforations extended to the apex of the canine. Adapted from Aboul-Ela et al. (115).

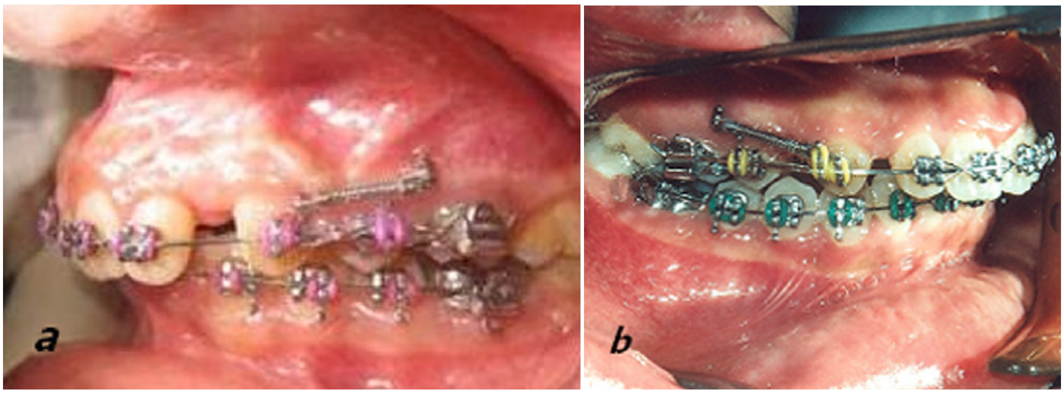

Figure 15: (a) Class I canine relationship achieved two months after retraction on the operated side; (b) Class I canine relationship not achieved on the non-operated side. Adapted from Aboul-Ela et al. (115).

Prospective animal research

Numerous animal studies have been conducted to examine the biological effects and processes involved with corticotomies. Duker (117), in 1975, duplicated Köle's technique in a report on alveolar corticotomies using beagle dogs. Vertical buccal corticotomies and horizontal bicortical osteotomies 5 mm above maxillary root apices were performed prior to orthodontic tooth movement over an 8 - 20 day period. Teeth were moved a distance of 4mm and pulp vitality and healthy periodontal tissues were maintained. By using only labial and lingual corticotomy cuts to circumscribe the roots of the teeth, Generson et al. (118) revised Köle's technique and reported successful results with a one-stage corticotomy-only technique without the supra-apical osteotomy.

Cho and co-workers (119) extracted the second bicuspids from two beagle dogs and, after four weeks of healing, performed corticotomies on the buccal and lingual side of the alveolar bone in the right quadrants of the jaw. Twelve perforations of the buccal and lingual cortical plates were performed at 3 mm intervals. All third bicuspids in both jaws were moved mesially by a 150 g force using NiTi coil spring with/without guiding wire. After 8 weeks of orthodontic movement, the authors reported that there was approximately four times greater movement on the corticotomy side of the maxilla and approximately two times as much tooth movement on the corticotomy side of the mandible.

Iino et al. (93) found that orthodontic tooth movement increased for at least two weeks after the corticotomies in beagle dogs and explained that the rapid alveolar bone reaction in the bone marrow cavities led to less hyalinization of the periodontal ligament on the alveolar wall. Hyalinization of the periodontal ligament at the compression side was observed only at the first week, while on the control side, it was observed throughout the experiment at one week, two weeks, and four weeks.

An animal study by Mostafa (116) found that the corticotomy-facilitated (CF) technique accelerated tooth movement two-fold. Perforations were made into the cortical bone and miniscrews were placed as skeletal anchors for the distalisation of first premolars. The study also showed greater osteoblastic activity on the compressive side in the orthodontics-only group, as the osteoblasts presumably attempt to reverse the resorption of the alveolar bone, hindering further tooth movement. On the tension side, osteogenesis was more active in the CF group because of more extensive stretching of the periodontium from the faster tooth movement. The quality of the bone in the CF group was found to be lamellar, whereas in the control group the bone was the woven type with wide marrow spaces. The author suggested that this may result in a greater relapse tendency in the control group.

Sanjideh and co-workers (120) extracted the mandibular third and maxillary second premolars of five foxhounds, and, in a split mouth study, aimed to determine whether corticotomy procedures increased tooth movement and also whether a second corticotomy procedure after four weeks affected the rate of tooth movement. Orthodontic forces were applied for a total of eight weeks. Tooth movement in areas of the single corticotomy procedure were approximately twice as high as the control side (2.4 mm vs. 1.3 mm). In the areas that had two corticotomy procedures, there was significantly greater tooth movement than in areas with the single corticotomy procedure, though differences were limited and of questionable clinical significance (2.3 mm vs. 2.0 mm). However, a higher rate of tooth movement was maintained over a longer duration of time in areas where the second corticotomy procedure was performed.

Baloul and co-workers (121) examined 114 Sprague-Dawley rats in three treatment groups (selective alveolar decortication alone; tooth movement alone; and "combined" therapy). The surgical procedure involved five decortication dots on the buccal and palatal aspects of the maxillary first molar tooth and mesial tooth movement was produced with an orthodontic force of 25 g. The orthodontic force was applied for up to 42 days and the specimens were examined with radiographic, tomographic, and molecular methods. Baloul and co-workers concluded that, at seven days, there was statistically significantly increased tooth movement and decreased bone volume. There was no significant difference in bone mineral density between the groups. After 14 days, the bone volume fraction in the combined treatment group (corticotomy and tooth movement) was statistically significantly greater. Additionally, they found that RNA markers of both osteoclastic cells and osteoblastic cells were raised, indicating that there was increased osteoclastogenesis and anabolic activity in response to alveolar decortication and tooth movement. Baloul and co-workers (121) concluded that the "alveolar decortication enhances the rate of tooth movement during the initial tooth displacement phase; this results in a coupled mechanism of bone resorption and bone formation during the earlier stages of treatment, and this mechanism underlies the rapid orthodontic tooth movement".

The rat model was used to evaluate the changes in cytokine expression associated with corticotomies (122). Forty-eight adult rats were divided into four treatment groups: orthodontic forces only (50 cN); soft tissue flap combined with orthodontic forces; orthodontic forces combined with a soft tissue flap and three cortical plate perforations; and an untreated control group. The orthodontic forces displaced the maxillary first molar in a mesial direction. The corticotomy combined with the orthodontic tooth movement resulted in statistically significantly greater magnitude of tooth movement compared with the other groups. This group also showed the highest number of osteoclasts and greatest amount of bone remodelling. Of the 92 examined, the levels of 37 cytokines increased in the experimental groups, with the greatest increase seen in the corticotomy group. Although the levels of cytokine expression increased, the patterns of cytokine expression did not, which is consistent with the acceleration of processes involved in RAP (rather than creation of new processes).

In summary, the experimental literature of case reports, prospective human trials, and animal studies show that corticotomy combined with orthodontic tooth movement is able to increase the rate and magnitude of tooth movement significantly. However, the majority of the evidence consists of case reports, many with limited numbers, and animal studies. Additionally, the animal studies tend to utilise mesial orthodontic movements, which are not representative of all clinical situations.

As the understanding of the biological processes involved with acceleration of tooth movement improves, the corticotomy procedure has similarly evolved to become less invasive with less morbidity. This is a significant advancement from the original corticotomy procedure involving bony blocks (100). Modifications have included the addition of perforations into the cortical plate (94), elimination of the sub-apical cuts (123), abolition of the lingual vertical cortical cuts (58), and use of perforations only (without vertical cuts) (124).

Prospective clinical trials

Fischer (124) performed unilateral corticotomy procedures on impacted maxillary canines requiring surgical exposure, with the control side receiving routine surgical exposure. The corticotomy consisted of circular holes mesial and distal to the impacted tooth, approximately 2 mm apart. Comparisons of the two methods in six consecutively treated patients revealed a reduction of treatment time of 28–33% for the corticotomy-assisted canines. No differences were observed in the final periodontal condition between the canines exposed by these two methods.

In 2007, Lee et al. (125) compared the treatment outcomes of 65 adult females who were diagnosed with bimaxillary dentoalveolar protrusion. The subjects were treated by conventional orthodontics; corticotomy-assisted orthodontics in the maxilla and anterior segmental osteotomies in the mandible; or anterior segmental osteotomies in the maxilla and mandible. The corticotomy-assisted/segmental group completed treatment 8 months faster than the conventional orthodontic group and showed a more posteriorly positioned mandible with less mandibular incisor retroclination. The amount of change in the upper lip projection and angulation was greater in the corticotomy group compared with the group that underwent conventional orthodontics alone.

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6. RATIONALE FOR THE CURRENT STUDY

Research questions

After reviewing the literature, there is an apparent need for further studies of the biological processes related to corticotomy-assisted orthodontics. In order to address some areas where further data are needed, a number of questions will be investigated:

- What bony changes occur following a flap procedure or corticotomy procedure?
- What bony changes occur following orthodontic tooth movement in a buccal direction either in isolation or in combination with a flap or corticotomy procedure?
- Are there any regional bony changes distant to the area of tooth movement, flap surgery, or corticotomy?
- Is there evidence of the regional acceleratory phenomenon following tooth movement, flap surgery, corticotomy, or tooth movement combined with flap surgery or corticotomy?

Aims/objectives of the project

The aims of this study were to validate the local and regional biological response to pre-orthodontic corticotomy in an animal model; to evaluate the local and regional bone response to a flap procedure with and without tooth movement; and to evaluate the local and regional bone response to a corticotomy procedure with and without tooth movement.

Hypotheses

The following hypotheses were proposed:

- Minimal bony changes relating to bone mineral density and bone fraction occur after flap surgery
- Significant bony changes relating to bone mineral density and bone fraction occur after corticotomy surgery
- Significant bony changes relating to bone mineral density and bone fraction occur after orthodontic tooth movement
- Greater bony changes relating to bone mineral density and bone fraction occur following increasing injury (through surgery or tooth movement) to the region of bone, especially with combinations of treatment
- Bony changes will be detectable in regions of bone distant from the area of surgery or orthodontic movement

Significance/contribution to the discipline

Periodontally Assisted Osteogenic Orthodontics (PAOO) has been performed in clinical practice for the past 10 years using the protocol popularised by the Wilcko brothers. It has been claimed in case reports that this interdisciplinary team approach has the potential to accelerate orthodontic tooth movement; with some authors claiming it to be three to four times quicker than traditional orthodontic tooth movement. Decreased treatment time may provide greater motivation for adult patients. More importantly, some authors consider that corticotomy facilitated treatment may offer an alternative to orthognathic surgery with less morbidity and mortality. In addition, these techniques could also reduce root resorption and provide a more stable result than traditional tooth movement alone.

However, the current evidence supporting PAOO is limited to case reports with a lack of experimental, animal-based histological studies, which are needed to elucidate the tissue changes associated with the technique. Hence, there is a void in the knowledge surrounding the biological response to PAOO. Additionally, most studies involving corticotomy have involved mesial/distal movement into extraction sites or intrusive movements for over-erupted molars. There are no published data on the effects of buccal/labial expansion on the periodontium involved in the animal model. This project is the first known study involving the radiographic assessment of the local and regional biological response associated with corticotomy-facilitated orthodontics in the buccal and palatal regions of bone. This differs from studies examining Surgically Assisted Rapid Palatal Expansion (SARPE), which involve Le Fort I osteotomy. Accelerated orthodontics is believed to be the consequence of corticotomy, which causes a demineralisation/ remineralisation process. This involves a dramatic increase in cortical bone porosity and trabecular bone surface turnover, which is the result of increased osteoclastic activity. This event was first observed and described by Frost as the “regional acceleratory phenomenon”. Combined with heavy orthodontic forces, the remaining, demineralised collagen matrix and islands of osteoid are transported with the root surfaces, resulting in “bone matrix transportation” with subsequent remineralisation of the bone in a new location. This study will allow us to elucidate the biology of tooth movement associated with this procedure (PAOO), the effect on teeth and bone, the magnitude of bony changes, and the regional bony changes related to treatment. The proposed research is expected to improve knowledge relating to this procedure, which is becoming more common in clinical practice.

7. STATEMENT OF PURPOSE

As presented in the previous literature review, there is limited biological data available. The current study attempts to investigate the bony changes associated with corticotomy and orthodontic tooth movement, using a rat model.

Therefore, the aim is to compare the bony changes in the buccal and palatal regions of bone associated with the maxillary first molar that is subjected to buccal orthodontic forces and/or corticotomy surgery. The orthodontic forces are applied over a seven day period. This would allow the validation of the local and regional biological response to pre-orthodontic corticotomy in an animal model. Additionally, evaluation of the local and regional bone response to a flap procedure with and without tooth movement and a corticotomy procedure with and without tooth movement would be performed.

The results of the study are presented in the following two papers, which have been prepared in the style of “Archives of Oral Biology”:

8. ARTICLE 1

Buccal bony changes following buccal orthodontic movement with or without corticotomy

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Abstract

Objectives

To evaluate the bony changes in the buccal region of bone in untreated and buccal orthodontic tooth movement groups, with or without adjunctive flap surgery or corticotomy.

Methods

A total of 36 male Sprague-Dawley rats, six to eight weeks old, were included in three control groups (no surgery; flap surgery; corticotomy) and three tooth movement groups (appliance only; flap and appliance; corticotomy and appliance). For the corticotomy groups, a full-thickness flap was elevated and a horizontal sub-apical groove on the buccal bone was made, with extensions vertically, mesial to the maxillary right first molar. A fixed appliance with a 100 g NiTi spring was placed to produce buccal tooth movement over a total experimental time of seven days. Following sacrifice, the specimens were prepared and resin-embedded.

Microcomputed tomography scans were performed. From these, a region of interest was outlined to include the buccal bone surrounding the maxillary first molar and 500 μm mesial and distal from the widest part of the tooth structure. Bone thresholding using the CTan progam (Skyscan, Belgium) was used to exclude tooth structure from the analysis and the bone mineral density and bone fraction (BV/TV) were determined. The micro-CT scan was performed with hydroxyapatite phantoms of 250 mg HA/cm^3 and 750 mg HA/cm^3 with 0.5 mm aluminium filter and 22.2 μm resolution scan.

Results

Corticotomy in conjunction with buccal tooth movement results in statistically significant reductions in bone mineral density in the buccal region of bone compared with corticotomy surgery without tooth movement and also compared with the contralateral control side. Additionally, corticotomy in conjunction with buccal tooth movement results in significantly decreased bone fraction in the buccal region compared with orthodontic tooth movement alone.

Conclusion

Following corticotomy and seven days of buccal orthodontic tooth movement in the rat model, there was a significant reduction in bone volume fraction in the buccal region of bone compared with controls and other treatment groups. This suggests that corticotomy combined with orthodontics is able to accelerate the bone resorption and formation processes associated with tooth movement, which supports the clinical results observed in reports of corticotomy-assisted orthodontics.

Introduction

Orthodontic treatment involves the movement of teeth through alveolar bone using externally applied forces and results in a biological reaction within the dento-alveolar tissues. Hence, orthodontics is characterised as bone manipulation therapy, which provides the biomechanical and physiologic basis of orthodontics. The remodelling of bone following injury or stimulus involves a complex array of interwoven processes and these ultimately determine the rate of tooth movement. Several adjunctive treatment modalities have been examined in order to accelerate orthodontic tooth movement, including pre-orthodontic corticotomy.

A corticotomy is defined as a surgical procedure in which cortical bone is cut, perforated, or mechanically altered without involvement of the medullary bone. In laboratory studies, when a surgical incision was made into the head of the tibia in rabbits, new bone, including trabecular bone, formed around the incision area as a result of increased bone turnover (1, 2). Furthermore, orthodontists have long noted increased rates of tooth movement following orthognathic surgical procedures, though this effect is usually attributed to a postoperative acceleration of bone remodelling. A consequence of this observation is that maxillary corticotomy is now a routine procedure for surgically assisted rapid palatal expansion (3, 4). However, alveolar corticotomy to enhance the rate of tooth movement has developed more slowly, largely because of concerns about periodontal outcomes.

Surgical intervention to affect the alveolar housing and tooth movement has been described in various forms for over a hundred years, with Köle (5) in 1959 being the first to describe modern-day corticotomy-facilitated orthodontics. It was believed that the surgical preparation of the alveolus outlining ‘bone blocks’ would permit rapid tooth movement, as it was suggested that the dense layer of cortical bone offered the greatest resistance to tooth movement. Köle (5) used a combined interradicular corticotomy and supra-apical osteotomy technique for rapid tooth movement and, by the use of relatively gross movements accomplished with heavy orthodontic forces delivered by removable appliances, major active tooth movements were accomplished in 6 to 12 weeks. Numerous modified corticotomy techniques have been performed to potentially decrease treatment time (6-11).

Wilcko and co-workers (12) reported a revised corticotomy-facilitated technique that included periodontal alveolar augmentation, called accelerated osteogenic orthodontics (AOO) or periodontally AOO technique (PAOO). AOO involves a combination of “bone activation” (selective alveolar decortication, ostectomies, and bone thinning with no osseous mobilisation), alveolar augmentation using particulate bone grafting material, and orthodontic treatment. Case reports demonstrated acceleration of treatment, which reduced the usual overall treatment time by two-thirds. The rapid tooth movement associated with corticotomy-facilitated orthodontics was most likely the result of a demineralisation/remineralisation process consistent with the initial phase of the regional acceleratory phenomenon (RAP), namely an increase in cortical bone porosity and a dramatic increase of trabecular bone surface turnover due to increased osteoclastic activity (1).

Baloul and co-workers (13) examined cellular and osseous changes following orthodontic tooth movement and selective alveolar decortication in a rat model. Micro-computed tomography, Faxitron analyses, and quantitative real-time polymerase chain reaction to assess mRNA were used to examine samples that ranged up to a 42-day treatment period. The results showed that the combined intervention resulted in increased tooth movement at seven days compared with tooth movement alone, with significantly decreased bone volume and bone mineral content. It was concluded that the “alveolar decortication enhances the rate of tooth movement during the initial tooth displacement phase; this results in a coupled mechanism of bone resorption and bone formation during the earlier stages of treatment, and this mechanism underlies the rapid orthodontic tooth movement" (13).

Although there have been numerous reports examining tipping and torquing orthodontic movement in combination with corticotomy, no previous study has examined the buccal area of bone combined with buccally-directed orthodontic movement. The null hypothesis is that there are no differences in mean bone mineral density and bone fraction following orthodontic tooth movement, flap surgery, corticotomy, or combinations of orthodontic tooth movement and surgery compared with other treatment modalities or an untreated control. The aims of this study were to validate the biological response to pre-orthodontic corticotomy in an animal model; to evaluate the bone response to a flap procedure with and without tooth movement; and to evaluate the bone response to a corticotomy procedure with and without tooth movement.

Materials and Methods

ETHICS APPROVAL

Ethics approval was obtained from the University of Adelaide Animal Ethics Committee (Project no: M-2009-172 and M-2009-172B).

EXPERIMENTAL ANIMALS

A total of 36 male Sprague-Dawley rats, six to eight weeks old, were obtained from Laboratory Animal Services (University of Adelaide) with an average body weight of 261.5 g (range 169-367 g). The animals were housed in the Animal House facility of the Medical School of the University of Adelaide, where all live animal procedures, including treatment, orthodontic appliance placement, and animal sacrifice, were performed. A diet of commercially manufactured standard rodent pellets (Parastoc Feed, Ridley AgriProducts, Murray Bridge, Australia), chocolate spread, and water was supplied, *ad libitum,* for the duration of the experiment. The rats were weighed daily to ensure adequate nutrition and stable health throughout the experimental period.

EXPERIMENTAL PROTOCOL

All treatment procedures were performed on the right maxillary first molar. The contralateral left maxillary first molar was untreated and served as a control. Sprague-Dawley rats were treated according to the timeline shown in Figure 1.

Appliance insertion (n=18)

Sacrifice (n=36)

Surgical treatment (n=24)

↓ ↓

-3 -2 -1 0 1 2 3 4 5 6 7 (Day)

↑ ↑

Calcein

5 mg/kg (n=36)

Alizarin Red

30 mg/kg (n=36)

Figure 1: Experimental timeline

As part of experimental procedures that will be discussed in further research, bone labels were administered to the animals through the course of the observation period. The first bone label (Calcein at 5 mg/ml) was administered three days prior to the commencement of any of the interventions by intra-peritoneal injection under isofluorane vapour and oxygen inhalation anaesthesia. The second bone label (Alizarin Red at 30 mg/mL) was administered at day 5 under the same anaesthetic regimen (Figure 1).

STUDY DESIGN

Thirty-six rats were randomly assigned to one of six treatment groups (see Table 1).

Table 1: Study groups

Group	Appliance	Surgery
1	No	No
2	No	Flap
3	No	Corticotomy
4	Yes	No
5	Yes	Flap
6	Yes	Corticotomy

ANAESTHESIA

To facilitate procedures, the rats were initially sedated within a gas chamber that received a continuous dual flow of isofluorane and oxygen for several minutes. Depending upon the initial weight of the rat, the isofluorane concentration reading was set between 2.5% and 3.0%.

Deep anaesthesia was provided through intraperitoneal injection of a mixture of Hypnorm® (fentanyl citrate, 0.315 mg/mL and fluanisone 10 mg/mL; Janssen-Cilag Ltd, High Wycombe, UK), Hypnovel® (midazolam hydrochloride, 5 mg/mL; Roche, Berne, Switzerland), and sterile water in a 1:1:2 ratio. Additionally, each rat was administered Temgesic® (buprenorphine 0.3 mg/mL; Reckitt Benckiser Healthcare Ltd, Dansom Lane, Hull, UK) 0.05 mg/mL at 1 mL/kg bodyweight by intraperitoneal injection, as required.

APPLIANCE CONSTRUCTION

In order to fabricate a custom orthodontic appliance for each animal, polyvinylsiloxane impressions (Honigum, Gunz Dental, Australia) were taken of the maxillary arch under inhalational sedation (isofluorane and oxygen). Additionally, impressions were taken at Day 0 and at sacrifice in order to produce study models for macroscopic measurement of tooth displacement.

A stone model was cast from the polyvinylsiloxane impression and an orthodontic appliance was constructed (Figure 2). A band was fitted over the maxillary incisors and a 1.5 mm diameter, half-round wire (Dentaurum, Australia) was soldered to the band to act as the major connector. A plunger/tube (0.018 inch) configuration was soldered to the major connector and a 100 g NiTi push coil spring (GAC Australia, Australia) was compressed with the plunger. This was attached to the right maxillary first molar with a stainless steel 0.010 inch ligature (3M Unitek, Monrovia, USA). The ligature attaching the plunger to the right maxillary first molar was passed between the contact point of the first and second molars and twisted tightly. Composite resin (Neobond, Dentsply GAC International, Bohemia, NY, USA) was used to bond the remaining pigtail and plunger to the tooth for both retention and comfort purposes. The band was cemented onto the incisors with Unitek Multi-cure Glass Ionomer (3M Unitek, Monrovia, USA) and light-cured with a halogen curing light.

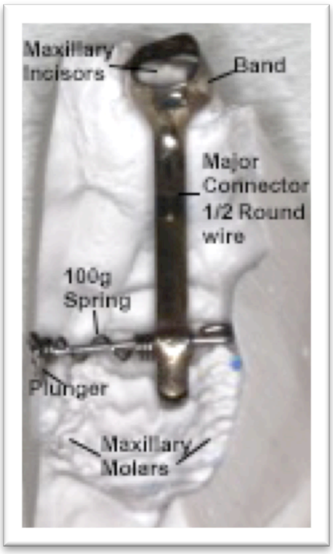


Figure 2: Appliance design

SURGICAL PROTOCOL

For the rats in the 'Flap Only' groups (Groups 2 and 5), a full-thickness mucoperiosteal flap was elevated on the buccal aspect of the right maxillary first molar. An intrasulcular incision was made using a scalpel blade along the buccal surface, extending anteriorly to the edentulous area mesial to the molar crown. A vertical incision was made between the roots of the first and second maxillary molars beyond the mucogingival junction. Following elevation of the flap, a tissue glue, GLUture (60% 2-octyl and 40% N-butyl cyanoacrylate, Abbott Laboratories, North Chicago, USA), was used to apposition the flap and promote healing by primary intention.

For the rats in the 'Corticotomy' groups (Groups 3 and 6), a full-thickness mucoperiosteal flap was raised using the same method as with the 'Flap Only' groups. A size 0.5 mm round, stainless steel bur (in a slow-speed handpiece) was utilised to create a trench in the cortical bone extending horizontally below the apices of the first molar and then vertically beyond the mesial root in an L shape. An isotonic saline irrigation was used to minimise bone overheating. The thickness and depth of the trench was the dimension of the bur tip. A tissue glue, GLUture, was used to apposition the flap and promote healing by primary intention.

ORTHODONTIC TOOTH MOVEMENT

For the 'Orthodontic Tooth Movement' groups (Groups 4, 5, and 6), the appliance was placed at Day 0 and acted upon the right maxillary first molar, exerting a buccal displacement force. The appliance remained in place and active for seven days until the animals were sacrificed.

SPECIMEN COLLECTION

At the completion of the observation period, the six groups of six animals were each euthanised with a lethal intraperitoneal injection of Lethabarb Euthanasia Injection (60 mg/mL with 1 mL/kg of a barbiturate derivative, Virbac, Australia). The maxilla was dissected out and trimmed to facilitate immersion fixation with 70% ethanol. Care was taken to avoid desiccation of tissues and to minimise damage to surrounding tissues during the embedding process. Tissue dehydration was carried out in 25 mL polypropylene tubes with a graded ethanol series prior to defatting with acetone and infiltration with methylmethacrylate. These processes occurred within a vacuum chamber as part of the processing protocol. Polymerisation of the methylmethacrylate took place in a 37°C oven for 2-3 days.

MICRO-TOMOGRAPHY

The SkyScan 1174 system (Skyscan, Kontich, Belgium), a desktop cone-beam X-ray scanner with a maximum potential spatial resolution of 3 microns, was used to examine the resin-embedded specimens and produce three-dimensional renditions of the teeth and their supporting bone. As bone density mapping was performed to display areas of demineralisation, two blocks of hydroxyapatite of known density were attached to the specimen during scanning for reference. The resin-embedded specimen was placed on a rotating platform within the scanner, in front of the X-ray source and rotated through 180 degrees. The raw data collected were reconstructed using SkyScan NRecon v1.4.4 software to provide an axial picture cross section. The software Dataviewer v1.4.4 was used to enable simultaneous visualisation of axial, coronal, and sagittal sections under interactive operator control. This allowed reorientation of the specimen and slices in three-dimensions in order to optimise the visualisation of the region of interest and its macroscopic structural features.

ANALYSIS OF TOMOGRAPHY

The tomography slices of 22.2 µm thickness were assessed using the CT Analyser program (version 1.12, Skyscan, Kontich, Belgium). The area of bone buccal to the maxillary first molar was selected as the region of interest for density analysis. Tomographic slices from the midpoint of the buccal root of the maxillary first molar to the buccal extent of the plate were selected and cropped to the region of interest. The dimensions of the region of interest were: 300 µm coronal to the most coronal cusp tip of the tooth; 300 µm apical to the most apical point of the sinus floor; 500 µm distal to the contact area with the maxillary second molar; and 500 µm mesial to the most mesial point of the mesial root apex. Using this template, the region was refined with manual relocation of pixel markers to specifically demarcate the borders of the buccal region of bone (Figure 3). Measurements were additionally determined using the known slice thickness (22.2 µm) and number of included slices. The region of interest was outlined at the buccal of both left (control) and right (test) maxillary first molar teeth.

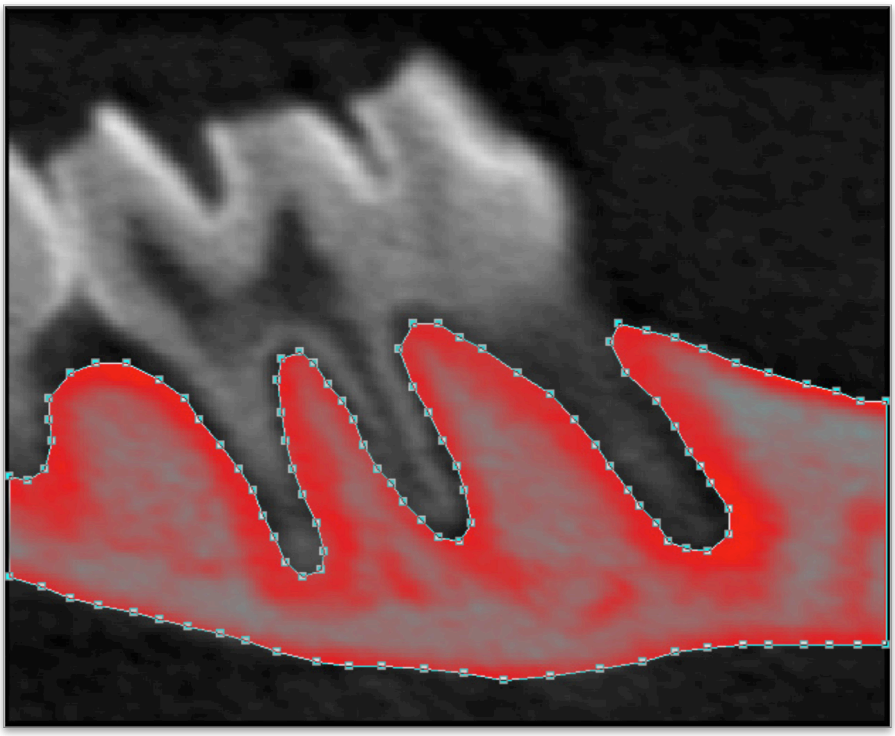


Figure 3: The region of interest of a single, sample tomographic slice (buccal region of bone). This sample slice was representative of one of the starting layers (as determined by visualisation of the buccal aspects of the buccal roots).

The program was used to determine the range, mean, and distribution of radiographic bone density (bone mineral density (BMD) and bone fraction) within the regions of interest (from the reconstructed slices).

VALIDATION

Two processes were undertaken to validate the obtained data. In order to confirm the accuracy of measurements using the microCT, radiographic measurements from the greatest point of convexity at the mesial surface of the maxillary first molar crown to the greatest point of convexity at the distal surface of the maxillary third molar crown were recorded using the Dataviewer program. These were compared with macroscopic measurements from photographic images of the sample measured with calipers. No significant differences were found between measurements from all paired samples (t-test).

Additionally, to determine whether there had been any changes in the alveolar crest height at the maxillary molars (relative to the cemento-enamel junction), the distance between the CEJ and bone crest was measured on the maxillary first molar using Adobe Photoshop at six points: midpoints of the mesiobuccal, buccal, and distobuccal roots, midpoint of the furcation areas, and the distal surface of the molar (see Figure 4). Using Dataviewer, the most convex point of the mesial surface of the first molar and the distal surface of the third molar were determined and the number of slides (in the program) between the two points was calculated. The number of slides was multiplied by 22.22 μm to determine the true distance between the two points. Using Adobe Photoshop CS5, the distance between the two points was measured and the scale was determined for each first molar sample. The distances between the CEJ and alveolar crest were proportioned based upon the measurements determined using the total distance of the three molar teeth.

A generalised estimating equation (GEE) was used to compare differences in the distance in different treatment groups, test and control sides, and area measured. A GEE approach was taken in order to account for clustering due to repeated measurements within the same mouth. In the model, side of arch, treatment group, area, and the interaction between treatment group and side of arch were included as predictor variables.

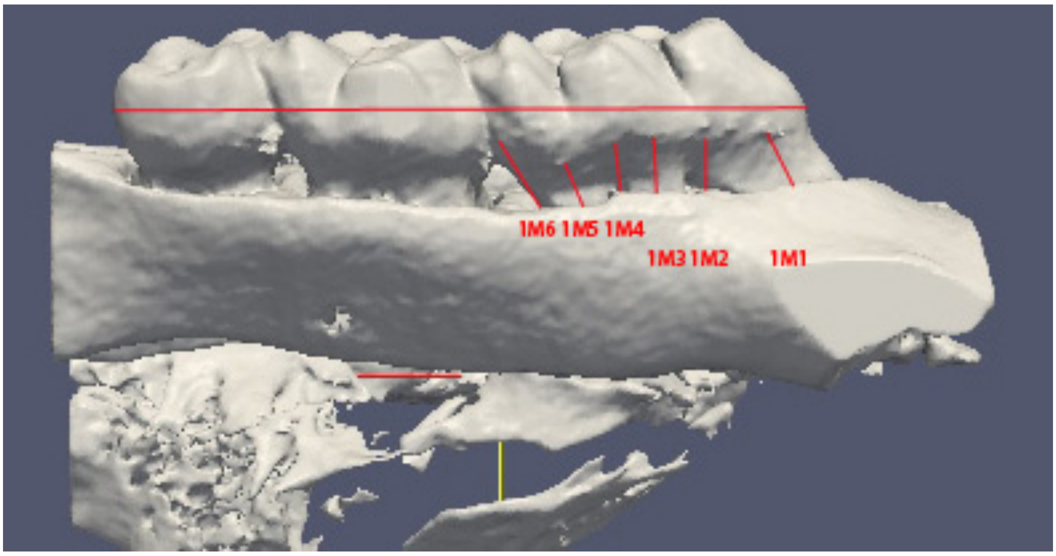


Figure 4: Points of measurement between the CEJ and alveolar crest. 1M1 to 1M6 represent the midpoints of the mesiobuccal, buccal, and distobuccal roots, midpoint of the furcation areas, and the distal surface of the molar, respectively. Lines from these points to the CEJ were measured to determine the distance of the alveolar crest to the CEJ. Image created using the Paraview program.

DATA ANALYSIS

To assess intra- and inter-examiner reliability, 11% of all specimens were randomly selected and remeasured. Intraclass correlation coefficients (type (3,1)), were used to test the intra- and inter-examiner reliability of bone mineral density and bone fraction measurements in the buccal regions (14). Calculations were performed using Stata Version 12. The intra-examiner reliability was greater than 0.998 and the inter-examiner reliability was greater than 0.996, which were considered to be acceptable (15).

A linear generalised estimating equation (GEE) was used to test for differences in bone mineral density and bone fraction between treatment groups and side of the arch (control and test). In the model, the treatment group, side of the arch, and the interaction between treatment group and side of arch were included as predictor variables. An independence working correlation matrix was used to adjust standard errors to account for the dependence in repeated observations from the same rat. Hence, a linear GEE was utilised to model the dependent data. A p-value <0.05 was considered to be statistically significant and all calculations were performed using SAS 9.3 (SAS Institute Inc., Cary, USA).

Results

HYDROXYAPATITE STANDARDS

In order to standardise the MicroCT data, two hydroxyapatite standard blocks were attached to the rat maxillae during scanning. The blocks were scanned in isolation, with differences shown in Figure 5. This range was due to variation in times of scanning and orientation of the standards. As a single scanning machine was used, with the same settings and scanning criteria, other variables were standardised. The data were aligned between the specimens to allow comparison and analysis.

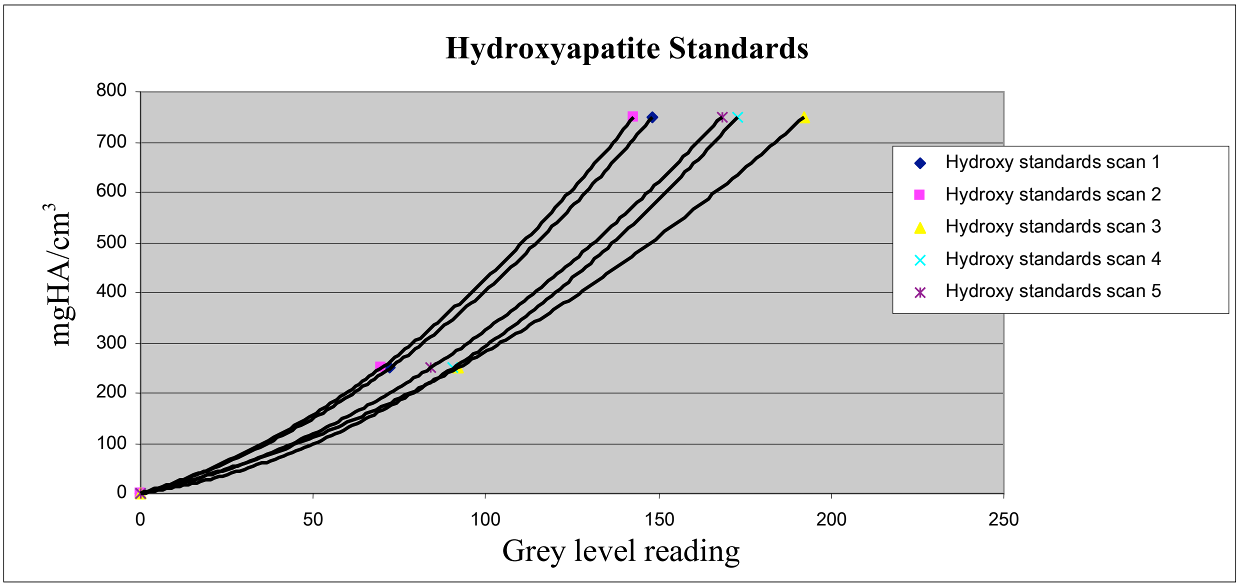


Figure 5: Graph showing the inter-scan differences of the same hydroxyapatite standards due to different times and orientation of the standards

STRUCTURAL CHANGES IN ALVEOLAR BONE

The overall bone structure and mineral content were examined by MicroCT. Representative MicroCT images of the buccal plate of bone associated with the first right molars are shown in Figure 6.

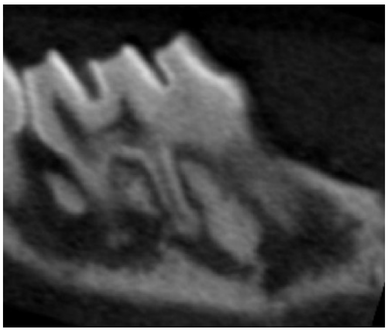


Figure 6: A sample MicroCT slice

Three-dimensional images of the sample were reconstructed using the Paraview program and the relationship between the buccal region of bone and the tooth roots confirmed (Figure 7). Macroscopic differences within the buccal region of bone between treatment groups could be seen in the reconstructed images (Figure 8), with the greatest changes visible in the combined 'Tooth Movement and Corticotomy' group (Group 6).

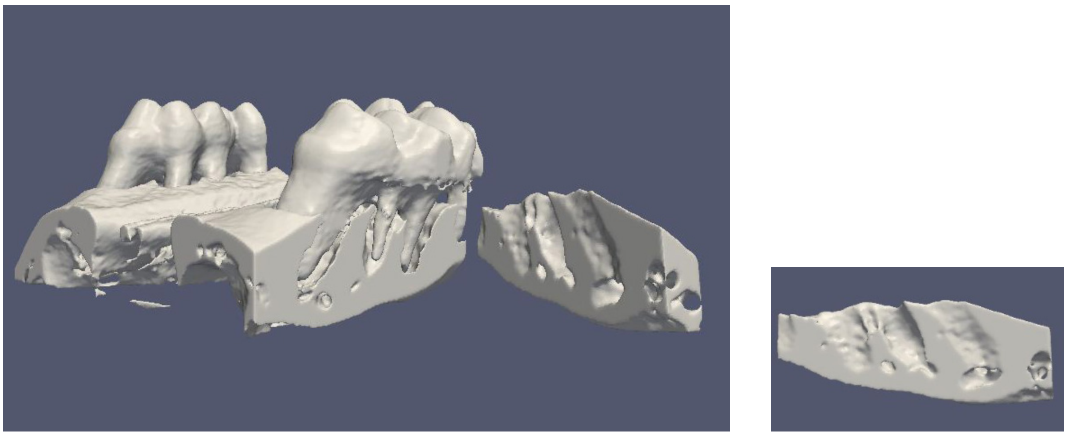
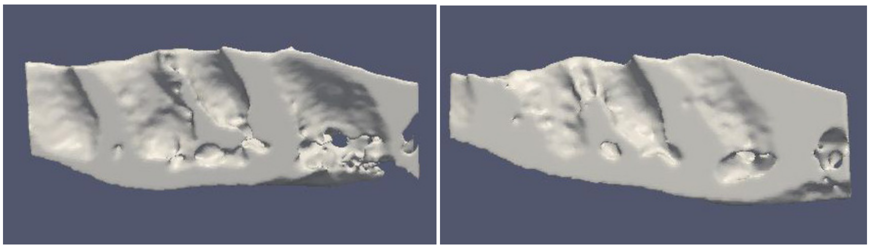
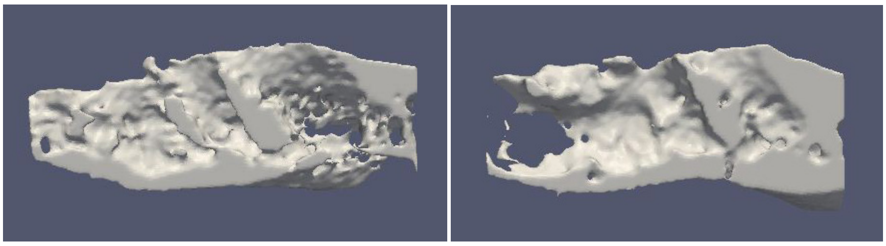


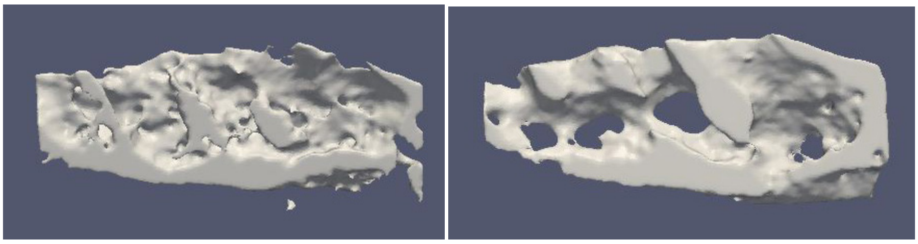
Figure 7: Reconstruction image showing the buccal plate in relation to the sample. Images created using the Paraview program.



No Treatment Flap Only



Corticotomy Only Tooth Movement Only



Tooth Movement + Flap Tooth Movement + Corticotomy

Figure 8: Sample reconstruction images of the buccal plate of bone, created using the Paraview program.

Radiographic measurements were validated by comparison of measurements from low-power photomicrographs of the specimens with measurements of the radiographic sections from the MicroCT. The macroscopic images were photographic images taken with a microscope. No significant difference was found between the macroscopic and radiographic measurements.

The distance between the cemento-enamel junction of the maxillary first molar and the crest of the alveolar bone was determined bilaterally at six points along the buccal surface. There were no significant differences in the distances within each treatment group when comparing the left (control) and right (test) sides, except within the ‘Flap Only’ group (Group 2). Similarly, when comparing the distance between the treatment sides of all groups, there were only significant differences when the ‘Flap Only’ group was compared with another group. All specimens within the ‘Flap Only’ group showed consistency within the measurements, with no outliers. The differences between the other groups were not statistically significant ($p>0.05$). Hence, there were no changes in the height of alveolar bone following the treatment intervention, except when the flap alone was performed.

BONE MINERAL DENSITY

There were no significant differences in mean buccal BMD between the control sides of all groups. Of all groups, only when tooth movement and corticotomy were combined (Group 6) was there a significant reduction in mean BMD compared with the contralateral control, suggesting that this treatment regime alone was sufficient to induce a significant change (Table 2). Other treatment modalities (Groups 1 to 5) showed no significant difference between treatment and the contralateral control mean BMDs after seven days (Figure 9). The mean buccal BMD of the treatment side was found to be reduced in all groups compared with the ‘No Treatment’ group (Group 1), with significant differences from the ‘Flap Only’, ‘Tooth Movement and Flap’, and ‘Tooth Movement and Corticotomy’ groups (Groups 2, 5 and 6) (Table 3). There was significantly greater reduction in mean buccal BMD when tooth movement and corticotomy (Group 6) were performed, compared with corticotomy alone (Group 3).

Table 2: Comparison of left side (control) and right side (test) in relation to mean bone mineral density

Mean Bone Mineral Density	Control (g/cm^3)	Test (g/cm^3)	P value
NO TREATMENT	1238.64	1246.78	0.8539
FLAP ONLY	1112.06	1077.23	0.1462
CORTICOTOMY ONLY	1208.6	1139.19	0.4358
TOOTH MOVEMENT ONLY	1201.42	1179.94	0.5528
TOOTH MOVEMENT + FLAP	1030.63	976.49	0.0603
TOOTH MOVEMENT + CORTICOTOMY	1189.35	1010.87	<0.0001

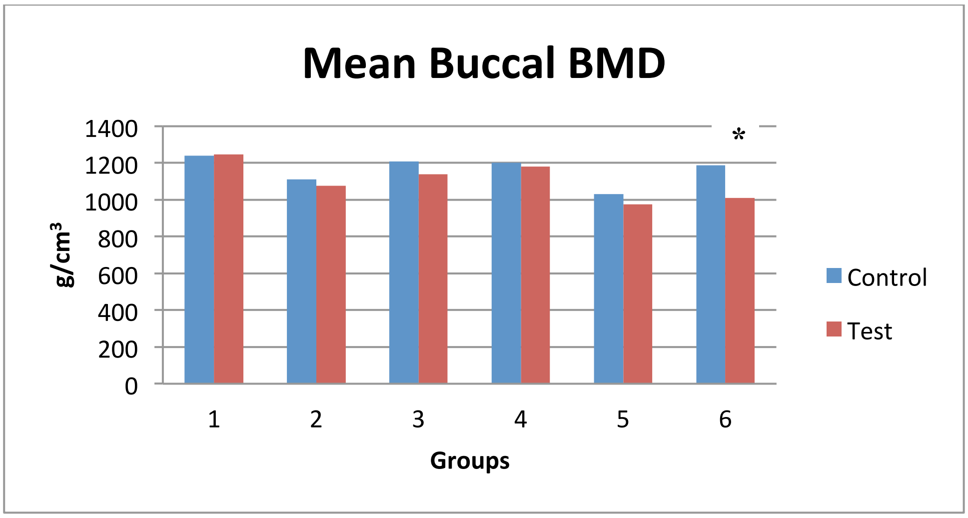


Figure 9: Graph comparing the left side (control) with the right side (test) in relation to mean bone mineral density. The study groups were as follows: Group 1- No Treatment; Group 2 - Flap Only; Group 3 – Corticotomy Only; Group 4 – Tooth Movement Only; Group 5 – Tooth Movement and Flap; and Group 6 – Tooth Movement and Corticotomy.

Table 3: P-values when comparing right side (test) groups in relation to mean bone mineral density

RIGHT (TEST) SIDE	NO TREATMENT	FLAP ONLY	CORTICOTOMY ONLY	TOOTH MOVEMENT ONLY	TOOTH MOVEMENT + FLAP	TOOTH MOVEMENT + CORTICOTOMY
NO TREATMENT		0.0046	0.1759	0.5331	0.009	0.0003
FLAP ONLY			0.0978	0.0154	<0.0001	<0.0001
CORTICOTOMY ONLY				0.9626	0.2487	0.0385
TOOTH MOVEMENT ONLY					0.1258	0.0047
TOOTH MOVEMENT + FLAP						0.0996

BONE FRACTION

Bone fraction, defined as the ratio of the segmented bone volume to the total volume of the region of interest (16), was significantly reduced in the treatment side for each group compared with the contralateral control side (Table 4 and Figure 10). However, for the control sides of the groups involving tooth movement (Groups 4, 5, and 6), there was a statistically significant reduction in mean bone fraction in comparison to 'No Treatment' (Group 1) despite these areas not having any local treatment. Additionally, the mean bone fraction in the control side of the 'Tooth Movement and Corticotomy' group (Group 6) was statistically significantly lower than the control side of all other groups.

For the treatment side, the tooth movement groups (Groups 4, 5, and 6) had statistically significant lower mean bone fraction compared with the 'Flap Only' group (Group 2). Compared with the right side of the 'No Treatment' group (Group 1), Groups 3, 4, 5, and 6 had statistically significantly lower mean bone fraction. The mean bone fraction of Group 6 was also statistically significantly reduced compared with Groups 3 and 4 (Figure 11).

Table 4: Comparison of left side (control) and right side (test) in relation to mean BV/TV

Bone Volume/Total Volume	Control (%)	Test (%)	P value
NO TREATMENT	70.6661	69.7407	0.4295
FLAP ONLY	68.3019	66.4325	0.0014
CORTICOTOMY ONLY	68.5950	60.5034	0.0046
TOOTH MOVEMENT ONLY	68.2737	60.3155	0.0023
TOOTH MOVEMENT + FLAP	67.5668	56.2852	<0.0001
TOOTH MOVEMENT + CORTICOTOMY	73.9069	52.9973	<0.0001

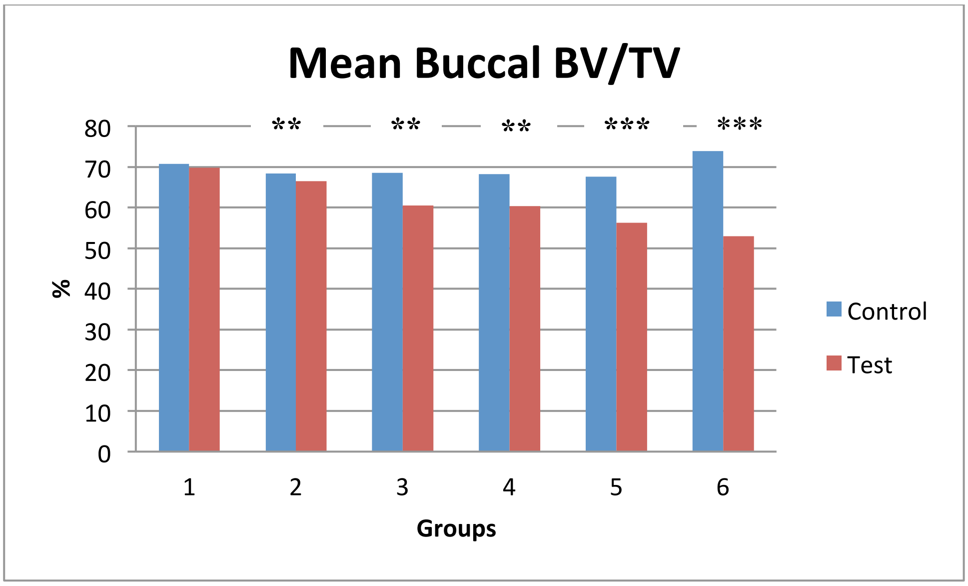


Figure 10: Graph comparing the left side (control) with the right side (test) in relation to mean BV/TV. (= p<0.05, **=p<0.01, ***=p<0.001). The study groups were as follows: Group 1- No Treatment; Group 2 - Flap Only; Group 3 – Corticotomy Only; Group 4 – Tooth Movement Only; Group 5 – Tooth Movement and Flap; and Group 6 – Tooth Movement and Corticotomy.*

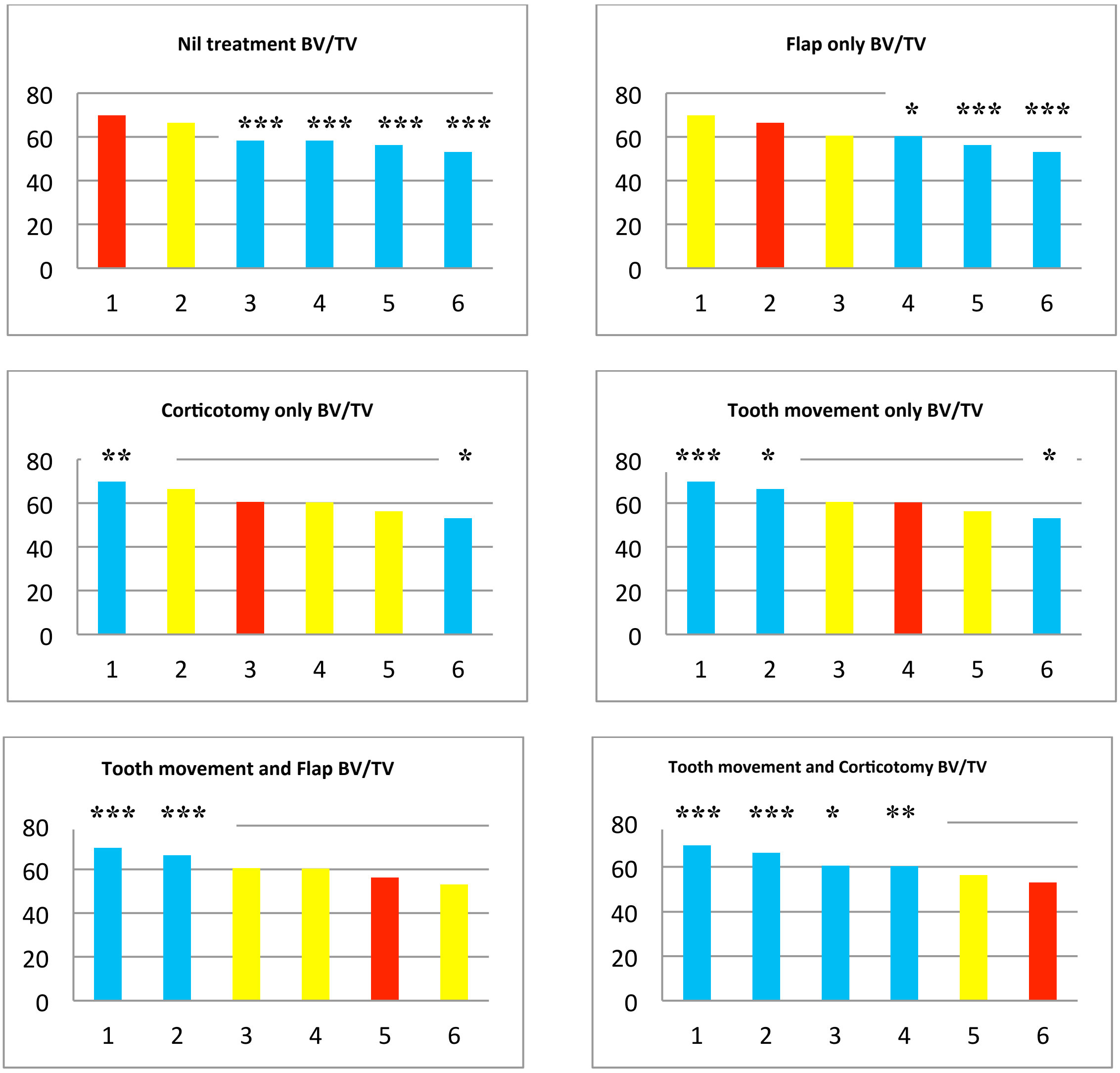


Figure 11: Graphs comparing the mean bone fraction of the treatment groups. Red bars represent the mean bone fraction of the baseline group. Yellow bars represent groups with mean bone fraction values not significantly different from the baseline group. Light blue bars represent groups with mean bone fraction values significantly different from the baseline group. (= $p<0.05$, **=$p<0.01$, ***=$p<0.001$). The study groups were as follows: Group 1- No Treatment; Group 2 - Flap Only; Group 3 – Corticotomy Only; Group 4 – Tooth Movement Only; Group 5 – Tooth Movement and Flap; and Group 6 – Tooth Movement and Corticotomy.*

Discussion

BONE MINERAL DENSITY

After seven days of treatment, the bone mineral density of the treatment side tended to decrease relative to the control side, though the differences were only statistically significant for the combined 'Tooth Movement and Corticotomy' group (Group 6, $p < 0.0001$). The BMD of the combined 'Tooth Movement and Corticotomy' group was also significantly different from the test sides of its control groups ('No Treatment' and 'Corticotomy Only'). This suggests that a sufficient degree of injury is required before a significant reduction in BMD will be detected.

Comparing the BMD of the regions of interest of the treatment side showed that there was a statistically significant reduction ($p=0.0046$) in the 'Flap Only' group compared with the 'No Treatment' group. When tooth movement was combined with the flap surgery (Group 5), the BMD was similarly statistically significantly different from the 'No Treatment' group as a greater degree of injury had been administered ($p= 0.0090$). These findings are consistent with the results from previous work by Yaffe and co-workers (17).

Although the BMD tended to reduce in the treatment side and was also statistically significantly lower in certain groups, the alveolar bone height did not change, except in the 'Flap Only' group. This indicates that there were no measureable vertical structural changes in the buccal plate of bone during the observation period. The changes in the alveolar bone height in the 'Flap Only' group are consistent with the findings of Yaffe and co-workers (17). Additionally, Tavtigian (18) showed that when a mucoperiosteal flap is raised, 0.46 mm of alveolar bone height is lost.

The contribution of the mucoperiosteal flap without the corticotomy procedure to PAOO was critically evaluated by Binderman and co-workers (19). They proposed that fiberectomy, produced by intrasulcular incisions around the teeth in the process of reflecting labial and lingual full-thickness flaps, was, by itself, a major signal for activating alveolar bone resorption, allowing PAOO. Yaffe and co-workers (17) suggested that the RAP bone remodelling phenomenon was involved, despite the absence of any surgical wounding of the cortical bone. The process involves a burst of osteoclastic activity and soft-tissue remodelling that resorbs the cortical lamina dura of the alveolar bone and thins the bone mass. This achieves osteopenia, making the bone more pliable to orthodontic force and helping to define the kinetics of tooth movement (19).

Additionally, it has been demonstrated that alveolar bone resorption commences only when the full-thickness flap surgery is performed by a coronal approach with a sulcular incision (20). In contrast, an apical surgical approach, which does not sever dentogingival and interdental Sharpey fibres, does not result in significant alveolar bone remodelling. It was considered that the mucoperiosteal flap elevation surgery could be separated into two procedures that have different biological effects on bone remodelling: surgical detachment of dentogingival and interdental fibres, producing a strong signal for osteoclastic alveolar bone resorption; and separation of mucoperiosteum from bone, which produces a burst of regional bone remodelling (regional acceleratory phenomenon) and reduction in bone density. However, other authors have suggested that the expressed level of RAP response does not surpass the threshold required to enable rapid tooth movement and, hence, cortical bone injury is essential (21, 22).

REGIONAL EFFECT

The presence of a regional effect was determined by comparing the bone mineral density and bone fractions of the left (control) side regions of interest. There were no statistically significant changes between groups in relation to BMD but there were significant reductions in bone fraction when the 'Tooth Movement Only' and 'Tooth Movement and Flap' groups were compared with the 'No Treatment' group. Additionally, the bone fraction of the control side of the combined 'Tooth Movement and Corticotomy' group was statistically significantly different from all other treatment groups. This is suggestive of a more systemic effect.

Baloul and co-workers (13) showed that surgical injury to the rat alveolus induced a dramatic increase in tissue turnover by the third week, which dissipated to a steady state by 11 weeks post-treatment. The effect of the injury was localised to the area immediately adjacent to the injury. Selective alveolar decortication was shown to induce a localised increased turnover of alveolar spongiosa, with the dramatic acceleration of demineralisation and remineralisation dynamics being the likely biological mechanism underlying the rapid tooth movement following corticotomy (21). This is suggestive of a regional acceleratory phenomenon.

BV/TV

A comparison of the treatment side of all groups showed a pattern of change in bone fraction consistent with the level of intervention involved. The mean bone fraction values for the buccal cortical plate of bone for each group reduced as the amount of injury increased. However, the bone fraction values were not statistically significantly different between all groups. Each group would have statistically significantly different values compared with other intervention groups with a great difference in injury. For example, the bone fraction of the 'No Treatment' group was statistically significantly different from all groups except for the ‘Flap Only’ group, which could be considered to be the next level of injury (relative to the other groups). The bone fraction of the ‘Corticotomy Only’ group was only statistically significantly different from the ‘No Treatment’ and combined ‘Tooth Movement and Corticotomy’ groups, suggesting that the level of effect of the corticotomy procedure is comparable to tooth movement combined with flap surgery. The bone fraction of the ‘Tooth Movement and Corticotomy’ group was statistically significantly reduced compared with all groups except for the ‘Tooth Movement and Flap’ group. All treatment groups showed statistically significantly reduced bone fraction in the treatment side compared with the contralateral control (untreated) side.

Previous studies have demonstrated that tooth movement with or without alveolar decortication is a coupled process in which there is bone resorption followed by formation during bone turnover (13). In their study, Baloul and co-workers examined 114 Sprague-Dawley rats in three treatment groups: selective alveolar decortication alone (SADc); tooth movement alone (TM); and “combined” therapy (SADc+TM). The surgical procedure involved five decortication dots on the buccal and palatal aspects of the maxillary first molar tooth. Mesial tooth movement was produced with an orthodontic force of 25 g. The orthodontic force was applied for up to 42 days and the specimens were examined with radiographic, tomographic, and molecular methods. Baloul and co-workers concluded that, at seven days, there was statistically significantly increased tooth movement and decreased bone volume. There was no significant difference in bone mineral density between the groups. After 14 days, the bone volume fraction in the combined treatment group (corticotomy and tooth movement) was statistically significantly greater. Additionally, they found that RNA markers of both osteoclastic cells and osteoblastic cells, such as macrophage colony stimulating factor, receptor activator of nuclear factor kappa-B ligand, osteoprotegerin, calcitonin receptor, tartrate-resistant acid phosphatase 5b, cathepsin K, osteopontin, bone sialoprotein, and osteocalcin, were raised. This indicated that there was increased osteoclastogenesis and anabolic activity in response to alveolar decortication and tooth movement. They concluded that the “alveolar decortication enhances the rate of tooth movement during the initial tooth displacement phase; this results in a coupled mechanism of bone resorption and bone formation during the earlier stages of treatment, and this mechanism underlies the rapid orthodontic tooth movement” (13).

Compared with the Baloul et al. study, the present study has several key differences. Although the specimens were examined at seven days, the entire follow-up period of the experiment in the Baloul study was 42 days (13). Additionally, the forces applied to the maxillary first molars acted in a mesial direction and the radiographic assessment examined the interradicular region of bone, not the buccal region as in this study. The buccal cortical plate of bone is a site of compression during buccal orthodontic tooth movement and comparatively, the interradicular area between the five roots of the molar includes areas of tension as well as compression. Baloul and co-workers also performed selective alveolar decortication involving five decortication dots buccal and palatal to the maxillary first molar, compared to the buccal L-shaped trough performed in this study. The effect of this difference is unknown, but because the regional acceleratory phenomenon is diffuse, the final acceleratory outcome may be comparable. There were no ‘Flap Only’ or ‘Flap and Tooth Movement’ groups in the Baloul study and they did not compare their treatment group results with any untreated teeth. However, they had a large sample size per group and they examined the specimens at a greater number of time intervals.

The results obtained in the present study are consistent with the findings of Baloul and co-workers (13), with a significant difference in bone fraction in the combined tooth movement and corticotomy group relative to the other treatment groups.

However, the regional acceleratory phenomenon commenced earlier with the combined group in the present study and it was suggested that the corticotomy procedure eliminates the lag phase of orthodontic tooth movement, resulting in more linear tooth movement (23).

In orthodontic treatment, increasing the magnitude of mechanical forces beyond the biologic threshold of the tissue to physiologically respond to the force leads to pathologic responses, such as cessation of tooth movement, root resorption, and hyalinization. In the study by Baloul et al. (13), the combined group showed a continuous and steady tooth movement from day 7 to day 21 without any evidence of a lag phase. It has been speculated that this different pattern of movement may have been due to less hyalinization of the periodontal ligament on the alveolar wall and the underlying mechanism of the combined technique was to 'bypass' the lag phase and initiate tooth movement earlier. The macroscopic tooth movement results from this study sample, as reported by Jong (23), showed that there was a statistically significant change in tooth position in the combined tooth movement and corticotomy group at day 7, with a magnitude greater than other tooth movement groups. Baloul and co-workers (13) suggested that there is possibly a 'coupling mechanism' that can be induced by alveolar decortication at an earlier time point without any pathological consequences. This is shown by the minimal change in overall bone mineral density and this mechanism underlies the rapid orthodontic tooth movement phenomenon. Resorption and apposition are not strictly sequential or independent events, but instead overlap in their action. This dynamic 'coupling' process suggests that the resorptive (osteoclastic) changes in the bone are accompanied by the formation (osteoblastic) process in healthy tissues during the same time window after mechanical injury (21).

The inclusion of multiple groups has allowed a graded assessment of the effect of injury to bone mineral density and bone fraction, and direct comparison with untreated groups and contralateral control sides of the jaw. One area of weakness in this study is the use of the rat model, as there are limitations in the use of models for the assessment of orthodontic tooth movement related to the differences in morphology and physiological processes in the rat skeleton compared with the human skeleton. In the adult human skeleton, the processes of modelling and remodelling are ongoing, independent, and have characteristic morphological features that can be differentiated histomorphometrically (24, 25). Modelling involves formation and resorption of bone occurring independently of each other. Hence, there are areas of activation and formation and other areas of activation and resorption. Comparatively, remodelling involves a coupling of bone resorption and formation, with a sequence of activation, resorption, then formation. In the rat skeleton, however, one of the processes is more prevalent than the other, depending on the age of the rat. There is a gradual transition from modelling to remodelling that is related to age progression in both cancellous and cortical bone (26).

Morphologically, there is a lack of Haversian remodelling in the rat skeleton, with cortical bone gain occurring in the periosteum and cortical bone loss at the endosteum (27). The alveolar bone in rats is generally denser than in humans and the bony plates are void of marrow spaces. This compares with the greater osteoid contact on the alveolar bone surfaces in humans (28). As the rat jaw is relatively small, secondary osteons are absent and marrow spaces are usually limited to the bone at the level of the apical third of the roots (29). This would suggest that a corticotomy procedure in the rat model would require a greater depth of cutting to reach the underlying medullary bone. The interradicular bone is predominantly woven or cancellous bone, composed of osseous trabeculae enclosing a network of vascular channels, some of which are continuous with the PDL (29). Hence, there is no distinct lamina dura detectable in radiographs.

The rat extracellular matrix has less mucopolysaccharides and calcium homeostasis is controlled by intestinal absorption rather than in the bone. Structural dissimilarities in the arrangement of the periodontal fibres and supporting structures have also been reported (30, 31).

Physiologically, although the principal mechanisms of tissue development during tooth formation in humans and rats are much the same, it occurs at a more rapid rate in rats (32). Bone turnover in the rat model is more rapid, with a reduction in the duration of each remodelling cycle in the alveolar bone of the adult rat mandible to an estimated six days (33). Due to the increased bone turnover, the phases involved in orthodontic tooth movement are similarly affected. It has been suggested that studies describing the characteristics and biological response in the linear phase of tooth movement should have an experimental period of at least two weeks (34).

Ideally, for studies in the rat model, the incisors should not be used for evaluation because they have a completely different morphology and they continuously erupt. This can affect the interpretation of data, because there can be poor anchorage for orthodontic tooth movement and reduced control of force direction. Hence, the use of molars is favoured, though it is important to be aware that there is a natural distal drift of rat teeth (27), which may lead to under- or overestimation of mesio-distal molar displacement. However, mesial orthodontic tooth movement is indicated compared with buccal tooth movement because there is a very limited amount of buccal bone. The bone is also more compact buccally than at the mesial side.

Another potential concern is the effect of the orthodontic appliance on occlusal loading due to the interference to function as a result of the orthodontic appliance. However, analysis of the untreated left maxillary first molars has shown that there were no adverse effects, with no significant decrease in alveolar bone mass or bone mineral density, which suggests minimally affected mastication or stress shielding.

This is the first reported study analysing the buccal cortical plate of bone and will be important for further assessment of the bony reaction to buccal orthodontic tooth movements, such as in expansion and proclination cases. Understanding of the biological mechanisms underlying the bony changes and tooth movement dynamics are essential for streamlining current and new orthodontic treatment modalities.

Further investigations into this sample group will be reported with histological assessment of the bone markers. Future research should examine animals treated with similar protocols but with a longer treatment/observation period (longitudinal) and an increase in sample size. Addition of osseous grafting could be performed to mimic the recently described clinical procedures, though the graft could have stimulatory (confounding) effects on the surrounding tissues. Other changes could include improvements in the appliance design and consideration of other animal models.

In conclusion, following corticotomy and seven days of buccal orthodontic tooth movement in the rat model, there was a significant reduction in bone volume fraction in the buccal region of bone compared with controls and other treatment groups. This suggests that corticotomy combined with orthodontics is able to accelerate the bone resorption and formation processes associated with tooth movement, which supports the clinical results observed in reports of corticotomy-assisted orthodontics. Therefore, the hypotheses of this study were supported. Further investigation is required and histological examination of the samples is currently underway.

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9. ARTICLE 2

Palatal bony changes following buccal orthodontic movement with or without corticotomy

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Abstract

Objectives

To evaluate the regional bony changes in the palatal region of bone in untreated and buccal orthodontic tooth movement groups, with or without adjunctive flap surgery or corticotomy.

Methods

A total of 36 male Sprague-Dawley rats, six to eight weeks old, were assigned to six control and treatment groups (no surgery; flap surgery; corticotomy; orthodontic appliance only; flap and appliance; corticotomy and appliance). For the surgery groups, a full-thickness flap was elevated. For the corticotomy groups, a horizontal sub-apical groove on the buccal bone was made, with extensions vertically, mesial to the maxillary right first molar. Over seven days, a NiTi spring on a fixed appliance provided 100 g of force, producing buccal tooth movement. The animals were sacrificed and the specimens were subsequently prepared and resin-embedded.
Microcomputed tomography scans were performed and a region of interest was outlined from these to include the palatal bone surrounding the maxillary first molar and 500 μm mesial and distal from the widest part of the tooth structure. Bone thresholding using the CTan progam (Skyscan, Belgium) was used to exclude tooth structure from the analysis and the bone mineral density and bone fraction (BV/TV) were determined. The micro-CT scan was performed with hydroxyapatite phantoms 250 mg HA/cm^3 and 750 mg HA/cm^3 with 0.5 mm aluminium filter and 22.2 μm resolution scan.

Results

Corticotomy in conjunction with buccal tooth movement results in no statistically significant difference in the bone mineral density of the palatal region of bone between treatment groups. However, there are variable results in relation to mean bone fraction. Changes in the structure of the palatal plate of bone in untreated contralateral areas were detectable, suggesting that a regional effect from pre-orthodontic corticotomy may be present.

Conclusion

There are osseous changes in the palatal region of bone in treated and untreated sites following corticotomy and seven days of buccal orthodontic tooth movement in the rat model. The changes suggest that there is a regional or systemic RAP effect that occurs as a result of an injurious stimulus.

Introduction

Orthodontic treatment involves the movement of teeth through alveolar bone using externally applied forces and results in a biological reaction within the dento-alveolar tissues, including the remodelling of bone. Remodelling following injury or stimulus consists of a complex array of interwoven processes and these ultimately determine the rate of tooth movement. Hence, there is a limitation to the rapidity at which orthodontic treatment can be completed without causing adverse effects. Several orthodontic procedures have been proposed recently to reduce overall treatment time and the theory of regional acceleratory phenomenon (RAP) of bone healing, though not a recent concept, has been brought back into the limelight.

The regional acceleratory phenomenon was first described by Frost (1) and is defined as 'a complex reaction of mammalian tissues to diverse noxious stimuli'. RAP affects an anatomical region, involving both the skeletal and soft tissues in an area, and the distribution reflects the regional vascular anatomy and innervation. Both the stimulated area and surrounding tissues are affected (2), such as the periodontium surrounding a tooth during orthodontic tooth movement, and, with severe stimuli, RAP can occur in contralateral regions of the body (1).

RAP is characterised by an acceleration of ongoing regional hard- and soft-tissue vital processes above normal response levels and may be considered to be a protective mechanism that evolved to potentiate tissue healing and to fortify local tissue immune reactions. Collectively, these accelerated processes represent the RAP and they include: growth of connective tissue structures (3, 4), remodelling of connective tissues (5), skin epithelialisation, soft tissue and bone healing, perfusion (6), and cellular turnover and metabolism (7). RAP does not seem to provide new processes but increases the rapidity of healing following bone fracture through all the post-fracture stages, including granulation, modelling, and remodelling (8). This results in healing occurring two to ten times more rapidly than otherwise, which means that additional remodelling cycles of resorption followed by formation are activated.

RAP is initiated when a regional noxious stimulus of sufficient magnitude affects the tissues. Frost (1) observed that the size of the affected region and the intensity of its response varied directly with the magnitude of the stimulus, though there was individual variation in the degree of the response. The noxious stimulus can greatly vary in nature and can include any perturbation of bone, including traumatic injuries, fractures (3), osseous surgery (9-12), vascular surgery, crushing injuries, thermal trauma, infections (13), and most non-infectious, inflammatory joint processes, including rheumatoid arthritis (7).

In a situation involving the fracture of bone, as was being examined by Frost (1) and Lee (14), the RAP response is divided into phases. The initial phase involves maximally stimulated bone formation, where woven or fibrous bone is produced to span a cortical gap (15). This bone is eventually remodelled into lamellar bone. This is then followed by a period of predominant resorption, where the medullary bone disappears and the number of osteoblasts decreases. This decreased regional bone density due to increased modelling space may also lead to regional tissue plasticity (1). The increased intracortical bone remodelling that is induced produces tunnelling within the cortex that can be seen on clinical radiographs. It is postulated that osteoclast and osteoblast cell populations shift in number, resulting in an osteopenic effect (2, 16).

Frost (17) estimated the total duration required for the remodelling – activation, resorption, and formation, to be 12 weeks. The duration of the RAP depends upon the severity of the stimuli, though in healthy humans, a single stimulus, such as a gunshot wound, will result in clinical evidence of RAP of approximately four months duration in bone (1). RAP begins within a few days of the fracture, typically peaks at one to two months, and may take six to more than 24 months to subside (8). The duration in soft tissues is shorter.

Although the concept of regional acceleratory phenomenon has been present for several decades, there have been few studies examining the microscopic changes associated with RAP and orthodontic tooth movement. Zaw and co-workers assessed osseous changes associated with the buccal region of bone following surgical treatment and buccal orthodontic tooth movement. However, the changes in the bone palatal to the molar tooth, away from the direction of tooth movement, have not been previously assessed. The null hypothesis was that there would be no osseous changes in the palatal region of bone of the treated and untreated molars following buccal orthodontic tooth movement and/or surgical treatment. The aims of this study were to validate the regional biological response to pre-orthodontic corticotomy in an animal model; to evaluate the regional bone response to a flap procedure with and without tooth movement; and to evaluate the regional bone response to a corticotomy procedure with and without tooth movement.

Materials and Methods

ETHICS APPROVAL

This project was approved by the University of Adelaide Animal Ethics Committee (Project no: M-2009-172 and M-2009-172B).

EXPERIMENTAL ANIMALS

Thirty-six male Sprague-Dawley rats, obtained from Laboratory Animal Services (The University of Adelaide), were used in this study. The rats were approximately six to eight weeks old, with an average body weight of 261.5 g (range 169-367 g). All rats were housed and all experiments, including surgical procedures, orthodontic appliance placement, and sacrifice, were performed in the Animal House facility of the Medical School of the University of Adelaide. A softened diet of commercially manufactured standard rodent pellets (Parastoc Feed, Ridley AgriProducts, Murray Bridge, Australia), chocolate spread, and water was provided for the duration of the experiment. The rats were weighed daily to ensure adequate nutrition and stable health throughout the experimental period.

EXPERIMENTAL PROTOCOL

The right maxillary first molar was used for the experimental procedures, while the contralateral left maxillary first molar was used as a control. The treatment timeline is shown in Figure 1.

Appliance insertion (n=18)

Sacrifice(n=36)

Surgical treatment (n=24)

↓ ↓

-3	-2	-1	0	1	2	3	4	5	6	7 (Day)

↑ ↑

Calcein — Alizarin Red

5 mg/kg (n=36) — 30 mg/kg (n=36)

Figure 1: Experimental timeline

Bone labels were administered to the animals at day -3 (Calcein at 5 mg/ml) and on day 6 (Alizarin Red at 30 mg/mL), as part of experimental procedures. These labels will be examined in further research. The bone labels were administered by intra-peritoneal injection under 3% isofluorane vapour and oxygen inhalation anaesthesia.

STUDY DESIGN

The 36 rats were randomly assigned to one of six treatment groups (see Table 1).

Table 1: Study groups

Group	Intervention
1	Nil
2	Flap only
3	Corticotomy Only
4	Appliance only
5	Appliance and Flap
6	Appliance and Corticotomy

ANAESTHESIA

The animals were initially sedated within a gas chamber that received a continuous dual flow of isofluorane and oxygen for several minutes. The isofluorane concentration varied between 2.5% and 3.0% depending upon the initial weight of the animals.

For deep anaesthesia, an intraperitoneal injection of a mixture of Hypnorm® (fentanyl citrate 0.315 mg/mL and fluanisone 10 mg/mL; Janssen-Cilag Ltd, High Wycombe, UK), Hypnovel® (midazolam hydrochloride, 5 mg/mL; Roche, Berne, Switzerland), and sterile water in a 1:1:2 ratio was used. Each rat was also administered Temgesic® (buprenorphine 0.3 mg/mL; Reckitt Benckiser Health care Ltd, Dansom Lane, Hull, UK) 0.05 mg/mL at 1 mL/kg bodyweight by intraperitoneal injection, as required.

APPLIANCE CONSTRUCTION

A custom orthodontic appliance was designed for each animal using polyvinylsiloxane impressions (Honigum, Gunz Dental, Australia) of the maxillary arch under inhalational sedation (isofluorane and oxygen). The impressions were taken at Day 0 and also at sacrifice in order to produce study models for macroscopic measurement of tooth displacement.

Using the polyvinylsiloxane impression, a stone cast was made and an individualised orthodontic appliance was constructed (Figure 2). A metal band was fitted over the maxillary incisors and a 1.5 mm diameter, half-round wire (Dentaurum, Australia) was soldered to the band to act as the major connector. A plunger and tube (0.018 inch) configuration was soldered to the major connector and a 100 g NiTi push coil spring (GAC Australia, Australia) was compressed with the plunger. This was attached to the right maxillary first molar with a stainless steel 0.010 inch ligature (3M Unitek, Monrovia, USA). The ligature attaching the plunger to the right maxillary first molar was passed between the contact point of the first and second molars and twisted tightly. Composite resin (Neobond, Dentsply GAC International, Bohemia, NY, USA) was used to bond the remaining pigtail and plunger to the tooth for both retention and comfort purposes. The band was cemented onto the incisors with Unitek Multi-cure Glass Ionomer (3M Unitek, Monrovia, USA) and light-cured with a halogen curing light.

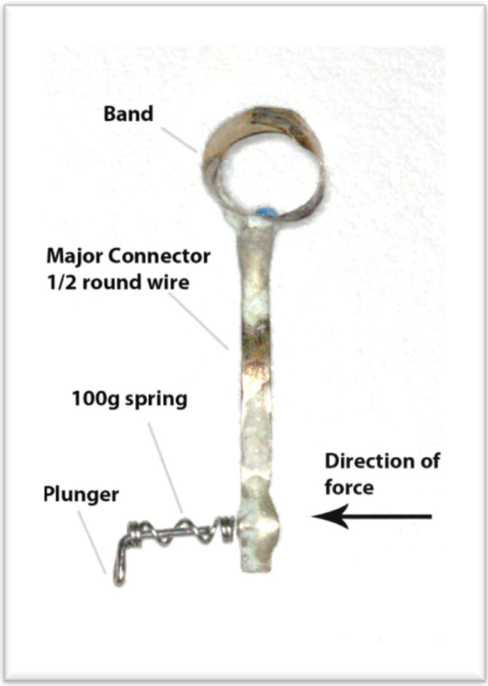


Figure 2: Appliance design

SURGICAL PROTOCOL

For the rats in the 'Flap Only' groups (Groups 2 and 5), a full-thickness mucoperiosteal flap was elevated on the buccal aspect of the right maxillary first molar with the aid of magnification. An intrasulcular incision was made using a scalpel blade along the buccal surface, extending anteriorly to the edentulous area mesial to the right maxillary molar crown. A vertical incision was made between the roots of the first and second maxillary molars beyond the mucogingival junction. Following elevation of the flap, a tissue glue, GLUture (60% 2-octyl and 40% N-butyl cyanoacrylate, Abbott Laboratories, North Chicago, USA), was used to apposition the flap and promote healing by primary intention.

For the rats in the 'Corticotomy' groups (Groups 3 and 6), a full-thickness mucoperiosteal flap was raised using the same method as with the 'Flap Only' groups. A size 0.5 mm round, stainless steel bur (in a slow-speed handpiece) was utilised to create a trench in the cortical bone extending from apical to the apices of the first molar horizontally and mesially to beyond the mesial root in an L shape. An isotonic saline irrigation was used to minimise bone overheating. The thickness and depth of the trench was the dimension of the bur tip. A tissue glue, GLUture, was used to apposition the flap and promote healing by primary intention.

ORTHODONTIC TOOTH MOVEMENT

For the ‘Orthodontic Tooth Movement’ groups (Groups 4, 5, and 6), the appliance was placed on the right maxillary first molar at Day 0, exerting 100 g of buccal displacement force. The appliance was left in place for seven days until the animals were sacrificed.

SPECIMEN COLLECTION

At the completion of the observation period, the six groups of six animals were each euthanised with a lethal intraperitoneal injection of Lethabarb Euthanasia Injection (60 mg/mL with 1 mL/kg of a barbiturate derivative, Virbac, Australia). The maxilla was dissected out and trimmed to facilitate immersion fixation with 70% ethanol. Care was taken to avoid desiccation of tissues and to minimise damage to surrounding tissues during the embedding process. Tissue dehydration was carried out in 25 mL polypropylene tubes with a graded ethanol series prior to defatting with acetone and infiltration with methylmethacrylate. These processes occurred within a vacuum chamber as part of the processing protocol. Polymerisation of the methylmethacrylate took place in a 37°C oven for two to three days.

MICRO-TOMOGRAPHY

The SkyScan 1174 system (Skyscan, Kontich, Belgium), a desktop cone-beam X-ray scanner with a maximum potential spatial resolution of 3 microns, was used to examine the resin-embedded specimens and to produce three-dimensional renditions of the teeth and their supporting bone. As bone density mapping was performed to display areas of demineralisation, two blocks of hydroxyapatite of known density were attached to the specimen during scanning for reference. The resin-embedded specimen was placed on a rotating platform within the scanner, in front of the X-ray source and rotated through 180 degrees. The raw data collected were reconstructed using SkyScan NRecon v1.4.4 software to provide an axial picture cross section. The software Dataviewer v1.4.4 was used to enable simultaneous visualisation of axial, coronal, and sagittal sections under interactive operator control. This allowed reorientation of the specimen and slices in three-dimensions in order to optimise the visualisation of the region of interest and its macroscopic structural features.

ANALYSIS OF TOMOGRAPHY

The tomography slices of 22.2 μm thickness were assessed using the CT Analyser program (version 1.12, Skyscan, Kontich, Belgium). The area of bone palatal to the maxillary first molar was selected as the region of interest for density analysis. Slides were orientated so that only the palatal region of bone was included in the analysis to avoid inclusion of the interradicular region of bone (Figure 3). All slides from the palatal surface of the mesial root to the most palatal point of the mid-palatal root were included and then an additional 500 μm of palatal bone was included in the region of interest. The other dimensions of the region of interest were: 300 μm coronal to the most coronal cusp tip of the tooth; 300 μm apical to the most apical point of the sinus floor; 500 μm distal to the contact area with the maxillary second molar; and 500 μm mesial to the most mesial point of the mesial root apex. Using this template, the region was refined with manual relocation of pixel markers to specifically demarcate the borders of the palatal region of bone (Figure 4). Measurements were additionally determined using the known slice thickness (22.2 μm) and number of included slices. The region of interest was outlined at the palatal of both left (control) and right (test) maxillary first molar teeth.

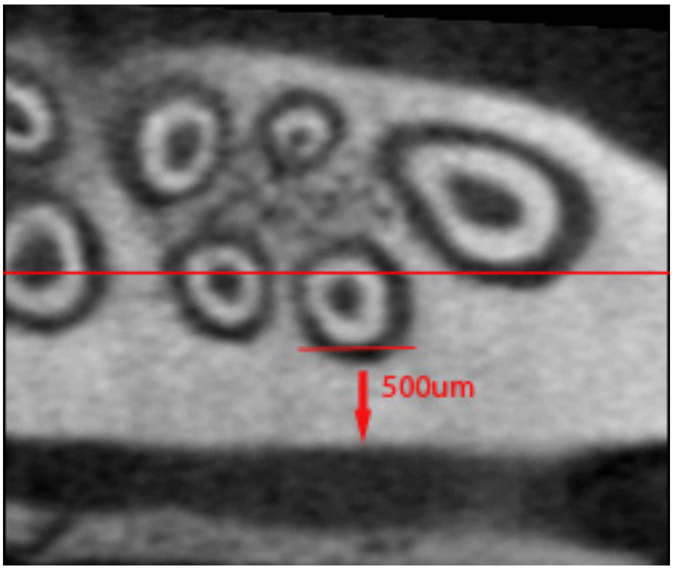


Figure 3: Determining the region of interest

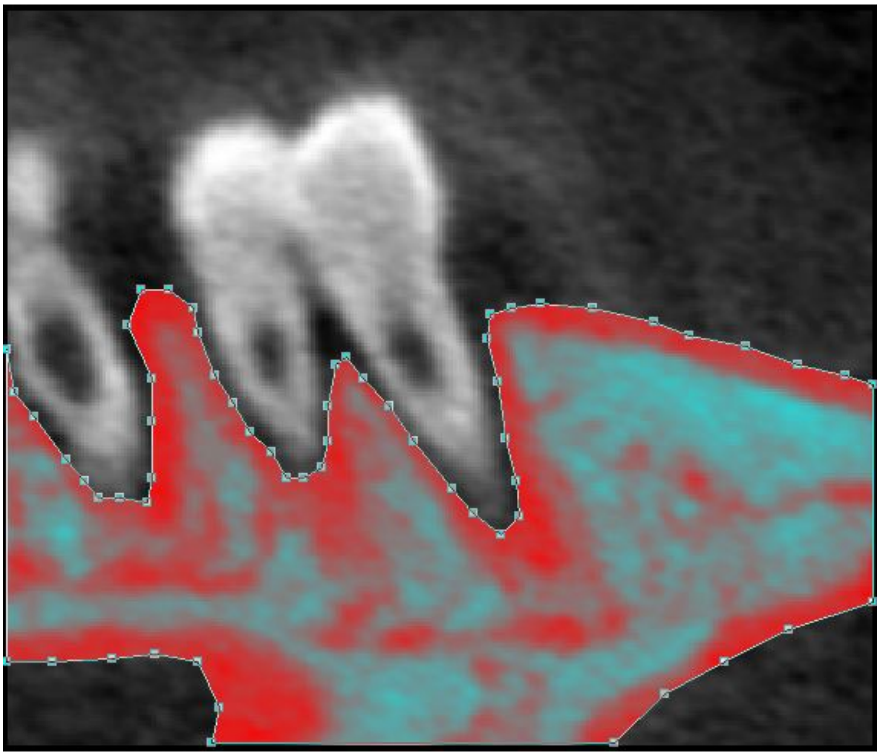


Figure 4: The region of interest (palatal region of bone)

The program was used to determine the range, mean, and distribution of radiographic bone density within the regions of interest (from the reconstructed slices).

DATA ANALYSIS

To assess intra- and inter-examiner reliability, 11% of all specimens were randomly selected and the buccal regions measured on two separate occasions. Intraclass correlation coefficients (type (3,1)) were used to test the intra- and inter-examiner reliability of bone mineral density (BMD) and bone fraction measurements in the buccal regions (18). Calculations were performed using Stata Version 12. The intra-examiner reliability was greater than 0.998 and the inter-examiner reliability was greater than 0.996, which were considered to be acceptable (19).

A linear generalised estimating equation (GEE) was used to test for differences in bone mineral density and bone fraction between treatment groups and side of the arch (control and test). In the model, the treatment group, side of the arch, and the interaction between treatment group and side of arch were included as predictor variables. An independence working correlation matrix was used to adjust standard errors to account for the dependence in repeated observations from the same rat. Hence, a linear GEE was utilised to model the dependent data. A p-value <0.05 was considered to be statistically significant and all calculations were performed using SAS 9.3 (SAS Institute Inc., Cary, USA).

Results

HYDROXYAPATITE STANDARD

The MicroCT data were standardised using two hydroxyapatite blocks that were attached to the rat maxillae during scanning. The blocks, which had two gradations of density (250 mg HA/cm^3 and 750 mg HA/cm^3), were scanned in isolation, with differences shown in Figure 5. The variation in time of scanning and orientation of the blocks produced the range of values. The single scanning machine was used for all specimens, with set parameters and scanning criteria. Other variables were standardised and hence the data between the specimens could be aligned for comparison and analysis.

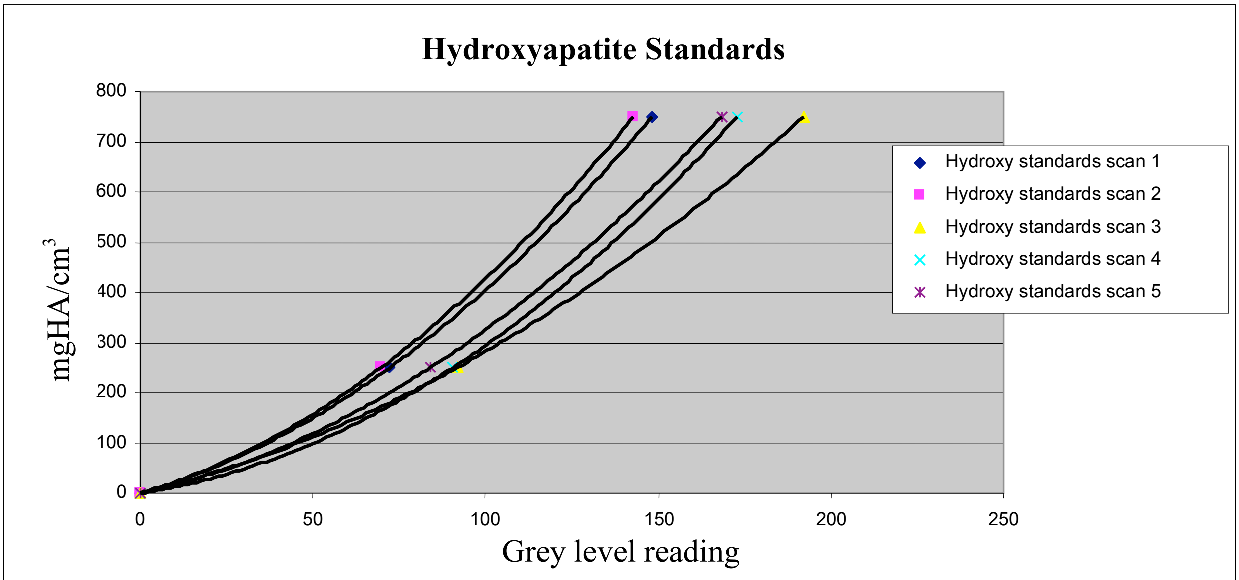
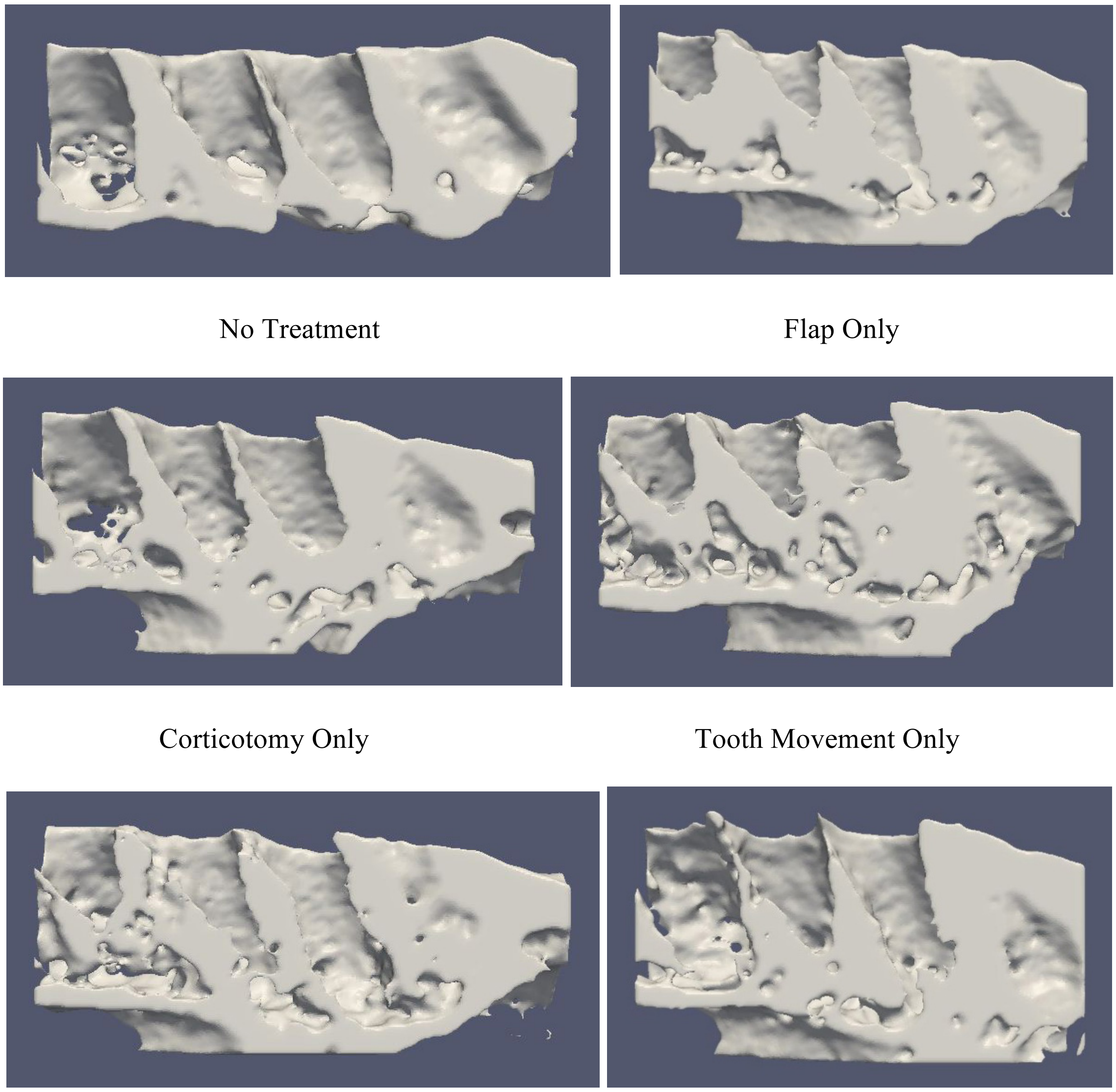


Figure 5: Graph showing the variation between scans of the hydroxyapatite standard block due to changes in time of scanning and orientation of the block

QUALITATIVE CHANGES IN ALVEOLAR BONE

Using MicroCT, the palatal region of bone associated with the upper first molars was examined. Changes in overall bone structure and mineral content were assessed and three-dimensional images of the samples were reconstructed using the Paraview program. Structural changes in the palatal region of bone between treatment groups were able to be assessed in the reconstructed images (Figure 6 and 7). In the images from the left (control) side of the palatal region of bone, greater mottling of the bone at the margins of the periodontal ligament space and within the body of the bony plate was present in the groups subjected to more invasive treatment (on the contralateral side). Similar changes were present in the images from the test side.



No Treatment — Flap Only

Corticotomy Only — Tooth Movement Only

Tooth Movement + Flap — Tooth Movement + Corticotomy

Figure 6: Sample reconstruction images of the palatal plate of bone from the untreated control side created using Paraview.

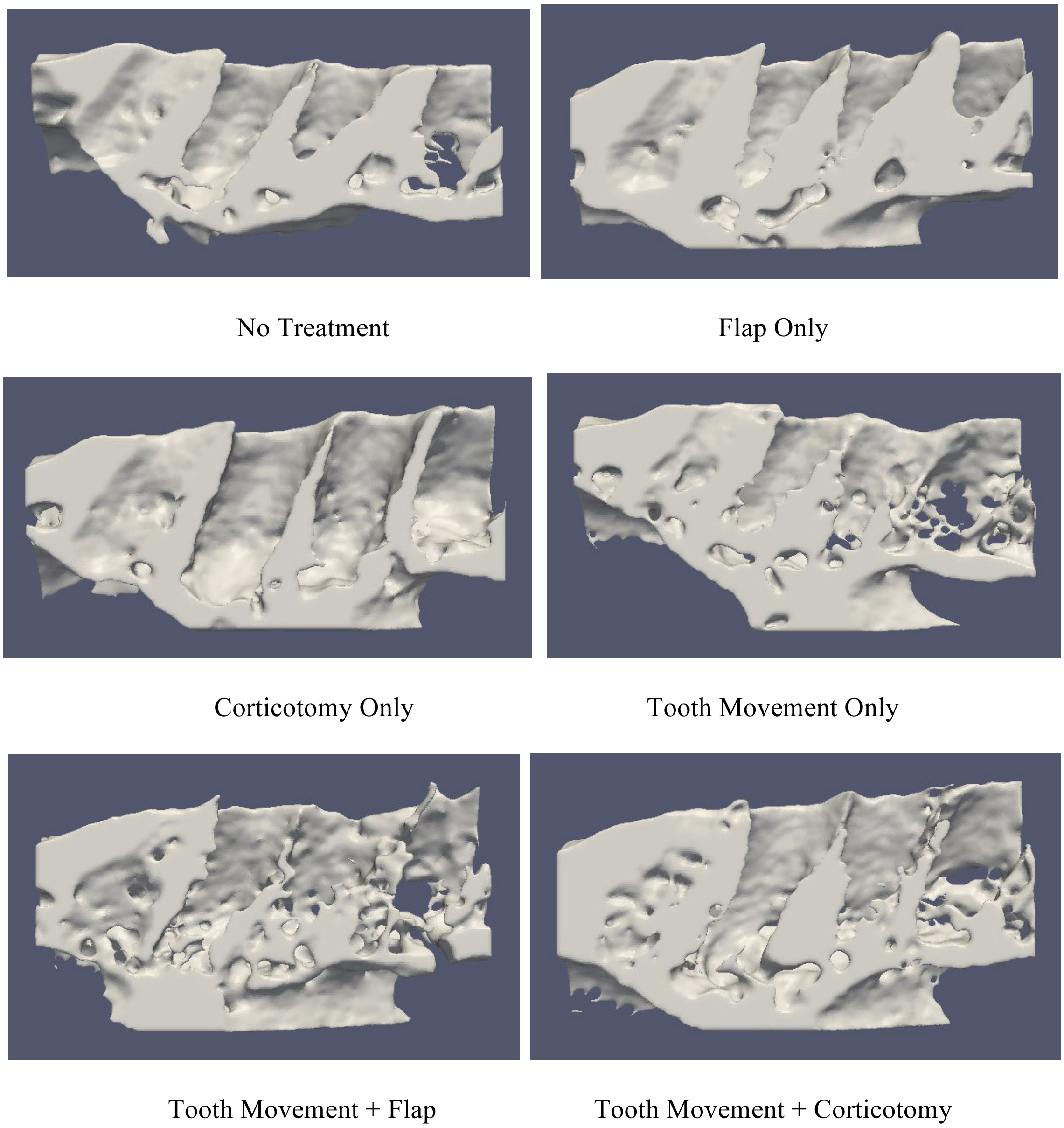


Figure 7: Sample reconstruction images of the palatal plate of bone from the test side created using Paraview.

MEAN BONE MINERAL DENSITY

There were no statistically significant differences in the mean bone mineral density values when comparisons were made between the different treatment groups on both the left (control) and right (test) sides (Figure 8). Additionally, there were no statistically significant differences when the control regions of the different groups were compared with each other and similarly for the test regions.

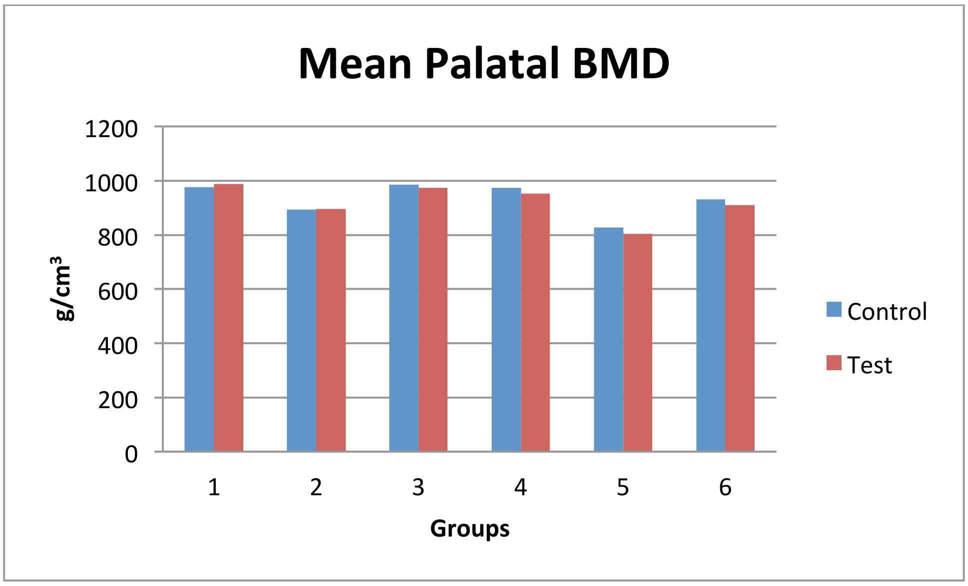


Figure 8: Graph comparing the left side (control) with the right side (test) in relation to mean bone mineral density. The study groups were as follows: Group 1- No Treatment; Group 2 - Flap Only; Group 3 – Corticotomy Only; Group 4 – Tooth Movement Only; Group 5 – Tooth Movement and Flap; and Group 6 – Tooth Movement and Corticotomy.

MEAN BONE FRACTION

Bone fraction is defined as the ratio of the segmented bone volume to the total volume of the region of interest (20). When comparing the control sides with the contralateral test sides, there were statistically significant reductions in bone fraction of the test side in the 'Corticotomy Only', 'Tooth Movement Only', 'Tooth Movement and Flap', and 'Tooth Movement and Corticotomy' groups (Groups 3, 4, 5, and 6) (Table 2). There were no statistically significant differences in bone fraction in the 'No Treatment' and 'Flap Only' groups (Groups 1 and 2) (Figure 9).

Table 2: Comparison of the left side (control) and right side (test) in relation to mean BV/TV

Bone Volume/Total Volume	Control (%)	Test (%)	P value
NO TREATMENT	57.4892	58.1218	0.4275
FLAP ONLY	60.3757	61.4466	0.0911
CORTICOTOMY ONLY	58.7796	55.4678	0.0003
TOOTH MOVEMENT ONLY	61.0244	54.4623	0.0002
TOOTH MOVEMENT + FLAP	57.4777	51.0062	0.0003
TOOTH MOVEMENT + CORTICOTOMY	56.1283	52.6727	0.0007

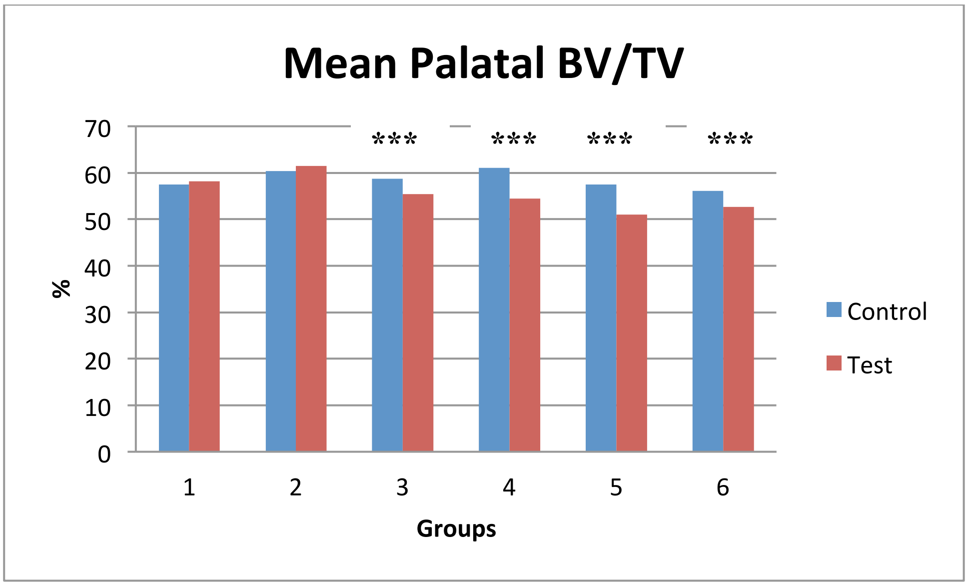


*Figure 9: Graph comparing the left side (control) with the right side (test) in relation to mean BV/TV (***= p<0.001). The study groups were as follows: Group 1- No Treatment; Group 2 - Flap Only; Group 3 – Corticotomy Only; Group 4 – Tooth Movement Only; Group 5 – Tooth Movement and Flap; and Group 6 – Tooth Movement and Corticotomy.*

When the mean bone fraction of the left, untreated sides was compared between groups, statistically significant differences were found (Table 3). The mean bone fraction of the 'No Treatment' group (Group 1) was statistically significantly lower than those of the 'Flap Only' and 'Tooth Movement Only' groups (Groups 2 and 4). Additionally, the mean bone fraction of the 'Tooth Movement and Corticotomy' group (Group 6) was statistically significantly lower than those of the 'Flap Only', 'Corticotomy Only', and 'Tooth Movement Only' groups (Groups 2, 3, and 4).

Table 3: P-values when comparing the left side (control) groups in relation to mean bone fraction

LEFT (CONTROL) SIDE	NO TREATMENT	FLAP ONLY	CORTICOTOMY ONLY	TOOTH MOVEMENT ONLY	TOOTH MOVEMENT + FLAP	TOOTH MOVEMENT + CORTICOTOMY
NO TREATMENT		0.0206	0.1589	<0.0001	0.9933	0.0556
FLAP ONLY			0.2344	0.6261	0.0855	0.0044
CORTICOTOMY ONLY				0.0289	0.3716	0.0307
TOOTH MOVEMENT ONLY					0.0143	<0.0001
TOOTH MOVEMENT + FLAP						0.3974

When the right (test) sides were compared, the mean bone fraction was statistically significantly greater in the 'No Treatment' and 'Flap Only groups (Groups 1 and 2) than in all other groups (Table 4). Additionally, the mean bone fraction of the

‘Corticotomy Only’ group (Group 3) was statistically significantly greater than that of the ‘Tooth Movement and Flap’ group (Group 5).

Table 4: P-values when comparing the right side (test) groups in relation to mean bone fraction

RIGHT (TEST) SIDE	NO TREATMENT	FLAP ONLY	CORTICOTOMY ONLY	TOOTH MOVEMENT ONLY	TOOTH MOVEMENT + FLAP	TOOTH MOVEMENT + CORTICOTOMY
NO TREATMENT		0.0103	0.0185	0.0144	<0.0001	0.0006
FLAP ONLY			<0.0001	<0.0001	<0.0001	<0.0001
CORTICOTOMY ONLY				0.05028	0.0065	0.0791
TOOTH MOVEMENT ONLY					0.0705	0.3387
TOOTH MOVEMENT + FLAP						0.4008

Discussion

Assessment of the reconstructed images of the palatal region of bone associated with the upper first molar revealed that there were macroscopic bony changes in the region of interest in both the control and test sides. There were areas of ‘mottling’ present at the margins of the PDL space and within the body of the bony plate, suggesting that increased remodelling of the bony architecture was occurring. Additionally, as the level of treatment/intervention that was performed on the contralateral side increased, the amount of bony change appeared to increase. As no treatment was performed on the left side, it could be considered that the changes were a response to the stimulation caused by treatment on the contralateral side. This could be attributable to a systemic acceleratory phenomenon (SAP). Alternatively, there could potentially be disuse atrophy of the control side due to compromised function and mastication as a result of obstruction by the appliance. This confounding factor was monitored through daily recording of rat weight to ensure adequate nutrition (and hence no reduction in function). Additionally, previous assessment of buccal cortical plate osseous changes by Zaw and co-workers found that there that there were no adverse effects, with no significant decrease in alveolar bone mass or bone mineral density. This suggested that mastication was minimally affected by the appliance.

Mueller and co-workers (21) examined the local healing of a bur hole bone defect made in the left tibia within the rat model. The subsequent local healing processes, as well as its possible impact on distant skeletal sites (tibiae, femora and the fourth lumbar vertebra) were assessed. As there were significant increases in various bony parameters, it was concluded that a SAP accompanies the RAP. However, it was also concluded that the SAP affects only the cancellous, but not the cortical bone compartment. Although the results of the present study similarly display systemic changes in the bone around the contralateral teeth, the changes were detected within the cortical bone, which is the predominant component of the palatal plate of bone within the rat model.

After seven days of treatment, no statistically significant changes were found in mean bone mineral density when comparisons were made between the control and test regions and also when different treatment groups were compared with each other. The regional effect of traumatic stimuli (RAP) would result in an acceleration of normal ongoing processes but may also result in a localised osteopenia. However, as no overall bone mineral content was lost from the region of interest, it may be that RAP involves the acceleration of remodelling processes (resorption and formation) and structural changes that redistribute the bone mineral within the region of interest (22).

The bone fraction was significantly different in Groups 3, 4, 5, and 6 when the control side was compared with the test side. Although bone fraction changes in the palatal bone are expected as a result of buccal orthodontic tooth movement (Groups 4, 5, and 6), with creation of the tension zone, bone fraction changes as a result of the corticotomy procedure alone (Group 3) were also found. Hence, the surgical trauma of a corticotomy procedure in the buccal plate of bone was sufficient to result in reactive bony changes on the palatal side.

The bone fraction in the test areas reduced in comparison with the control side in Groups 4, 5, and 6, though it could have increased due to bone formation in the tension zone of orthodontic tooth movement. This reduction in bone fraction could also have resulted from an increase in the size of the palatal region of bone (the region of interest) due to buccal movement of the tooth within the ridge of bone. This would increase the volume of bone with no relative increase in mineralised bone content (during the seven days of movement). Additionally, there could be an increase in the width of the PDL space, which would also result in a reduction in mean bone fraction. However, the mottling visible in the three-dimensional reconstruction of the palatal regions indicated that true osseous changes had occurred.

When comparing the bone fraction differences between palatal regions of the untreated side of different groups, as well as the treated side of different groups, the combinations that were statistically significantly different, varied. Although changes were evident between the groups, with six rats in each treatment group, it may be that individual variation played a major role. Frost (1) observed that the size of the region affected by RAP and the intensity of its response varied directly with the magnitude of the stimulus, but noted that there was individual variation in the degree of the response. It may be that the principal effect of RAP was concentrated at the site of injury and the regional or systemic effect could have been more greatly affected by an individual's inflammatory response. The differences between the rat model and human skeleton also need to be considered (23), especially with regard to the increased rate of bone metabolism and greater cortical bone proportion. As the rat molar and jaws are significantly smaller than human teeth and jaws, the relationship between interventions and bone reactions will also be affected (24). As the extent of RAP has not been well defined, the regional effects from the corticotomy procedure may affect the contralateral teeth and supporting bone in the rat model, rather than being a SAP effect. However, Sebaoun and co-workers (25) concluded that, within the rat model, the increased bone metabolism that occurred following a corticotomy procedure was localised to the area immediately adjacent to the site of injury and that there was negligible metabolic change across the dental arch or more than one tooth away. The variation in the morphology and physiological processes of the rat skeleton, in comparison to the human skeleton, can be considered to be an area of weakness in this study.

In conclusion, there were osseous changes in the palatal region of bone following corticotomy and seven days of buccal orthodontic tooth movement in the rat model. The changes occurred in both the control and test sides, suggesting that there was a regional or systemic RAP effect that occurred as a result of a stimulus. Thus, the hypotheses are supported. Further investigation is required and histological examination of the data is currently underway.

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10. CONCLUDING REMARKS

Although there are a growing number of studies examining corticotomy-assisted tooth-movement, the bulk of the evidence involves case reports and several animal studies. As the corticotomy procedure becomes more commonplace, there is greater importance in determining the underlying biological processes and potential outcomes and complications. Furthermore, randomised controlled clinical trials are needed to consider research questions related to rate of tooth movement, stability of treatment, adverse outcomes, morbidity of treatment, and patient acceptance.

Following corticotomy and seven days of buccal orthodontic tooth movement in the rat model, there was a significant reduction in bone volume fraction in the buccal region of bone compared with controls and other treatment groups. This suggests that corticotomy combined with orthodontics is able to accelerate the bone resorption and formation processes associated with tooth movement, which supports the clinical results observed in reports of corticotomy-assisted orthodontics. Furthermore, there were osseous changes in the palatal region of bone following the combined treatment, with changes occurring in both the control and test sides, suggesting that there is a regional and systemic RAP effect that occurs as a result of a stimulus. This is the first known reported study analysing the buccal and palatal cortical plates of bone and will be important for further assessment of the bony reaction to buccal orthodontic tooth movements, such as in expansion and proclination cases. Additionally, it adds to the body of evidence helping to decipher the biological mechanisms underlying the bony changes and tooth movement dynamics that are essential for streamlining current and new orthodontic treatment modalities.