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YEDITEPE UNIVERSITY
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CLINICAL AND HISTOMORPHOMETRICAL EVALUATION OF THE EFFECTS OF ALVEOLAR DECORTICATION AMOUNT ON ORTHODONTIC TOOTH MOVEMENT IN RATS

DOCTOR OF PHILOSOPHY THESIS

BEGÜM ASLAN, BDS

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SUPERVISOR
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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 12.06.2018 and numbered 2018/10-01

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BEYAN

Bu tezin kendi çalışmam olduğunu, planlanmasından yazımına kadar hiçbir aşamasında etik dışı davranışımın olmadığını, tezdeki bütün bilgileri akademik ve etik kurallar içinde elde ettiğimi, tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara kaynak gösterdiğimi ve bu kaynakları kaynaklar listesine aldığımı, tez çalışması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığını beyan ederim.

14.06.2018

Begüm ASLAN

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

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Begüm ASLAN

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LIST OF SYMBOLS AND ABBREVIATIONS

1,25 DHCC	1,25 Dihydroxycalciferol
AAO	Accelerated Osteogenic Orthodontics
ASA	Acetyl Salysilic Acid
ATP	Adenosine Triphosphate
cAMP	Cyclic Adenosine Monophosphate
CCL_2	Chemokine CC Ligand
cGMP	Cyclic Guanosine Monophosphate
CE	Coefficient of Error
CGMP	Cyclic Guanasine Triphosphate
CO_2	Carbondioxide
COX-2	Cyclooxygenase-2
EGF	Epidermal Growth Factor
Er, Cr: YSGG	Erbium, Chromium-Doped Yttrium, Scandium, Gallium And Garnet
Er:YAG	Erbium-Doped Yttrium Aluminium Garnet Laser
FGF	Fibroblast Growth Factor
Ga-Al-As	Gallium-Aluminum-Arsenide
He-Ne	Helium Neon
IL	Interleukin
LDLT	Low Density Laser Therapy
LFMV	Low Frequency Mechanical Vibration
M-CSF	Macrophage colony stimulating factor
Micro-CT	Micro Computed Tomography
MMP	Matrix Metalloproteinase
MOP	Micro-osteoperforation
mRNA	Messenger ribonucleic acid
Nd:YAG	Neodymium-Doped Yttrium Aluminum Garnet
NSAID	Nonstereoidal Anti-inflammatory Drug
NO	Nitric oxide

OC	Osteocalcin
OH:	Hydroxyl
OP	Osteopontin
OPG	Osteoprotegerin
PAOO	Periodontally Accelerated Orthodontics
PDL	Periodontal Ligament
PDGF	Platelet Derived Growth Factor
PGE_2	Prostaglandin E_2
PPI	Proton Pump Inhibitors
PRP	Platelet Rich Plasma
PRF	Platelet Rich Fibrin
PTH	Parathyroid Hormone
RANK	Receptor Activator of Nuclear Factor Kappa B
RANKL	Receptor Activator of Nuclear Factor Kappa B Ligand
RAP	Regional acceleratory phenomenon
Rln	Relaxin hormone
RNA	Ribonucleic acid
SAD	Selective Alveolar Decortication
SD	Standard deviation
TGF	Transforming Growth Factor
TRAP	Tartrate-resistant acid phosphatase
TNF	Tumor Necrosing Factor

ABSTRACT

Aslan, B. (2018). Clinical and Histomorphometrical Evaluation of the Effects of Alveolar Decortication Amount on Orthodontic Tooth Movement in Rats. Yeditepe University, Institute of Health Sciences, Department of Orthodontics, PhD thesis, İstanbul.

The aim of this study was to clinically and histomorphometrically investigate the effect of alveolar decortication amount, which was performed by slow handpiece transmucosally, on orthodontic tooth movement.

This study was conducted on 72 male Sprague-Dawley rats. The rats were divided into the following groups: control (orthodontic force only), 1D (single decortication) and 3D (three decortication) groups. In each group, 3 time points were studied: 7, 14 and 21 days. In all groups, the upper first molars were mesially moved by 50 gr-force by means of Sentalloy closed coil spring placed between the upper first molars and incisor teeth. In addition to this, in the experimental group animals, shallow bone perforations were made either only on the mesial (1D group) or on the mesial, palatal and buccal sides (3D group). Decortication process was performed using 0.8 mm diameter round burs with transmucosal approach. The animals were sacrificed on day 7, 14 and 21 according to their follow-up groups. The maxillae were dissected and prepared for histological examination. Maxillary impressions were taken prior to appliance placement (initial) and after appliance removal (final). Orthodontic tooth movement amount was measured on the dental casts made from initial and final impressions.

Orthodontic tooth movement measurements revealed no significant difference between the control and experiment groups on day 7, 14 and 21 ($p>0.05$). As a result of histological examination, no statistically significant difference was found between the groups in terms of BV/TV ratio, BVF and TVF ($p>0.05$) on day 7 and 14. On day 21, while BV/TV ratio ($p=0.015$) and BVF ($p=0.008$) parameters decreased significantly, TVF parameter ($p=0.01$) increased significantly in 1D group compared to control group. On day 7, mesial osteoclast numbers showed statistically significant increase in 3D group compared to 1D group ($p=0.037$). On day 14, mesial osteoclast numbers in 1D ($p=0.03$) and 3D groups ($p=0.001$) increased significantly compared to control group. On day 21, the increase was only seen in 3D group compared to control ($p=0.008$) and 1D groups

(p=0.025). In terms of distal osteoclast numbers, 3D group presented significant increase compared to control group on day 14 (p=0.007).

In this study, acceleration of orthodontic tooth movement with minimal invasive approach was aimed. Even though the osteoclast numbers increased following transmucosal decortication procedure, the results revealed that the procedure had no significant clinical effect on orthodontic tooth movement induced with 50 g of force in rats.

Key words: Rat, orthodontic tooth movement, alveolar decortication, accelerated tooth movement, stereology

ÖZET

Aslan, B. (2018). Sıçanlarda Alveolar Dekortikasyon Miktarının Ortodontik Diş Hareketine Etkilerinin Klinik ve Histomorfometrik Olarak İncelenmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Ortodonti ABD., Doktora Tezi. İstanbul.

Bu çalışmanın amacı, sıçanlarda yavaş tur motoruyla transmukozal olarak gerçekleştirilen alveolar dekortikasyon sayısının ortodontik diş hareketine etkilerinin klinik ve histomorfometrik olarak incelenmesidir.

Bu çalışma 72 erkek Sprague-Dawley sıçanı üzerinde yürütülmüştür. Sıçanlar şu şekilde gruplara ayrılmıştır: kontrol (sadece ortodontik kuvvet uygulaması), 1D (tek dekortikasyon) ve 3D (üç dekortikasyon). Her grup için 3 farklı zaman aralığı incelenmiştir: 7, 14 ve 21. günler. Tüm gruplarda üst 1. Molar dişler, keser dişler ile 1. Molar dişler arasında yerleştirilen Sentalloy kapalı yaylar yardımıyla 50 g-kuvvet altında meziyale hareket ettirilmiştir. Buna ek olarak, deney gruplarında sığ kemik perforasyonları sadece meziyalde (tek dekortikasyon grubu) ya da mezial, palatinal ve bukkalde (üç dekortikasyon grubu) oluşturulmuştur. Dekortikasyon işlemi 0.8 mm çapında yuvarlak frezler kullanılarak transmukozal olarak gerçekleştirilmiştir. Hayvanlar bulundukları takip gruplarına göre 7., 14. ve 21. günlerde sakrifiye edilmişlerdir. Üst çene örnekleri diseke edilmiştir ve histolojik incelemeye hazırlanmıştır. Üst çene ölçüleri aparey yerleşiminden önce (ilk) ve aparey çıkarılmasından sonra (son) alınmıştır. Ortodontik diş hareketi miktarı ilk ve son ölçülerden elde edilen alçı modeller üzerinde ölçülmüştür.

Ortodontik diş hareketi miktarı ölçümleri 7., 14. ve 21. günlerde kontrol ve deney grupları arasında anlamlı bir farklılık olmadığını göstermiştir ($p>0.05$). Histolojik değerlendirmeler sonucunda 7. ve 14. günlerde gruplar arasında BV/TV, BVF ve TVF açısından anlamlı bir farklılık bulunmamıştır ($p>0.05$). 21. günde, 1D grubunda kontrol grubuna kıyasla BV/TV oranı ($p=0.015$) ve BVF parametresi ($p=0.008$) anlamlı olarak azalırken TVF parametresi ($p=0.01$) ise anlamlı olarak artmıştır. 7. günde 3D grubundaki meziyal osteoklast sayıları 1D grubuna göre anlamlı bir artış göstermiştir ($p=0.037$). 14. günde, 1D ($p=0.03$) ve 3D ($p=0.001$) gruplarındaki meziyal osteoklast sayıları kontrol grubuna göre anlamlı artış göstermiştir. 21. günde ise, bu artış kontrol ($p=0.008$) ve 1D ($p=0.025$) gruplarına kıyasla sadece 3D grubunda gözlenmiştir. Distal osteoklast sayıları

açısından, 14. günde 3D grubu kontrol grubuna göre anlamlı artış sergilemiştir (p=0.007).

Bu çalışmada minimal invaziv yaklaşımla ortodontik diş hareketinin hızlandırılması amaçlanmıştır. Transmukozal dekortikasyon işlemini takiben osteoklast sayıları artmış olsa da, sonuçlar bu işlemin sıçanlarda 50 gr-kuvvet uygulandığında ortodontik diş hareketi üzerinde anlamlı bir klinik etkisinin olmadığını ortaya koymuştur.

Anahtar kelimeler: Sıçan, diş hareketi, alveoler dekortikasyon, hızlandırılmış diş hareketi, stereoloji

1.INTRODUCTION and PURPOSE

Orthodontic force causes reactions in the teeth and tissues surrounding the teeth. Tooth movement is characterised with remodelling of dental and paradental tissues including pulp, periodontal ligament, alveolar bone and gingiva (99). These structures exhibit macroscopic and microscopic changes under mechanical forces with different magnitude, frequency and duration. Due to orthodontic force, the alteration in periodontal vascularity and blood flow leads to expression of various key molecules as cytokines, neurotransmitters, growth factors, colony stimulating growth factor and arachidonic acid metabolites (5). These molecules create suitable micro-environment for deposition and resorption in bone surrounding the teeth. Orthodontic tooth movement, which relies on simultaneous deposition and resorption mechanisms, is considered to be controlled by resorptive activity and thus osteoclastic activity (53).

Various clinical and *in vitro studies* have been conducted focusing on accelerating orthodontic tooth movement. These interventions can be categorised as injection of chemical agents (67,68,127-29,136,153-61), mechanical and physical stimulations (191-97), electromagnetic stimulations (200-205), gene stimulation (187-89), laser biostimulation (206-19) and surgical approaches (236-52, 283-90). Since injection of chemical agents can have local and systemic side effects, mechanical and physical methods are not economically feasible, the attention has been drawn to surgical methods recently.

The basis of surgical methods is interrupting the continuity of the cortical layer, which is resistant to tooth movement. Based on this principle, bone is surgically stimulated, the inflammation cascade is initiated and consequently, osteoclastogenesis is induced leading to a decrease in bone density and acceleration in tooth movement. In other words, surgical wounding of the bone gives rise to regional acceleratory phenomenon (RAP) and thus orthodontic tooth movement is accelerated (126, 136).

Osteotomies, corticotomies, dentoalveolar distraction osteogenesis and selective alveolar decortication are among the procedures to surgically facilitate tooth movement. Even if the methods are effective, however, reflection of mucoperiosteal flaps and bone cuts render these techniques highly invasive and ends up with low patient acceptance. Therefore, flapless techniques with higher patient acceptance are developed to accelerate orthodontic tooth movement.

In 2006, Park et al. (275) introduced corticision technique based on accelerating tooth movement by making cuts through the gingiva with surgical blade and hammer transmucosally. Later in 2009, Dibart et al. (264) introduced the piezocision technique, in which corticision cuts were made going through the buccal vertical cuts by means of ultrasonic wave. This technique also enabled soft and hard tissue grafting by tunnel approach.

Another minimally invasive technique called micro-osteoperforations was introduced by Alikhani et al. (280) in 2013. The method was based on making shallow perforations in the cortical bone transmucosally and stimulating regional demineralization-remineralization process to accelerate orthodontic tooth movement. The authors performed the micro-osteoperforations via new device by PROPEL Orthodontics and stated that this was a safe and effective method in accelerating orthodontic tooth movement.

The aim of this study was to evaluate the clinical and histomorphometrical effects of transmucosal alveolar decortication amount, which is made by slow handpiece, on orthodontic tooth movement in rats. We hypothesized that even small amount of surgical trauma in the bone could stimulate regional acceleratory phenomenon and accelerate orthodontic tooth movement. Our null hypothesis was that this procedure would not make any change on the rate of orthodontic tooth movement.

2. LITERATURE REVIEW

2.1 Biological Basis of Orthodontic Tooth Movement

2.1.1. Cells Involved in Tooth Movement

2.1.1.1 Fibroblasts

Fibroblasts are the main and the most abundant cells of connective tissue of periodontal ligament (PDL). Long and oval shaped fibroblasts are aligned along the periodontal fibres. They play role in collagen synthesis and lysis of mature collagen fibres via phagocytosis (11). They take their major role in *remodeling* of collagen fibres in PDL. Fibroblasts are responsible of maintaining, reconstruction and regeneration of PDL and alveolar bone structure (18). Therefore, any systemic condition or disease to cause dysfunction in fibroblasts results in rapid destruction of tooth supporting tisssues (19).

PDL fibroblasts are large cells containing organelles (granulated endoplasmic reticulum, golgi complex and many granulles) related with protein synthesis and their release. Content of organelles in cytoplasm of fibroblasts indicate synthetic and secretuary capacity of the cell (19).

Fibroblasts are found in active or inactive form in the matrix. Inactive fibroblasts (fibrocytes), are long cells containing less amount of cytoplasm while active fibroblasts are oval-shaped cells with more cytoplasm. Besides, actin form of cytoskeleton enables these cells to move freely during connective tissue synthesis and wound healing.

PDL fibroblasts respond to orthodontic force as follows: They activate stress sensitive calcium permeable channels and increase actin polymerisation. This leads to increase in C phosphate secretion. As a result, proliferation and differentiation of fibroblasts are stimulated.

Apart from synthesizing collagen, they also synthesize biologically active molecules such as plasminogen activator and inhibitor, proteinases, cytokines (PGE-2, IL-6) and growth factors (19).

2.1.1.2. Osteoblasts

Osteoblasts are differentiated from osteoprogenitor cells of mesenchymal origin. In bone tissue, they are responsible of expression, storage and mineralization of extracellular matrix. They are in charge of expression of non-collagen proteins of bone matrix (11) and synthesizing collagen type I with fibroblasts (9). Also, it has been reported that they express regulatory cytokines and growth factors (12). The number of osteoblasts decrease by age.

Histologically osteoblasts are recognized as a single layered of cuboidal, large cells.

Ninety percent of bone matrix consists of type I collagen whereas the remaining 10% consists of non-collagen proteins including osteocalcin, bone sialoprotein, osteopontin, osteonectin, fibronectin, proteoglicans. Among these proteins, expression of osteocalcin and bone sialoprotein are relatively limited by osteoblasts. This explains the use of osteocalcin and bone sialoprotein as osteoblastic *diagnostic marker* in most researches (11).

Osteoblasts produce organic matrix at a rate of 2-3 μm per day. Since these cells express high alkaline phosphatase activiy, bone mineralization occurs at a rate of 1-2 μm per day (13).

2.1.1.3. Osteocytes

Osteoblasts, which function during bone formation, form osteocytes by being embedded in the mineralized bone structure. Osteocytes that maintain their vitality for many years are the most abundant cells in mature bone. The nutrition of osteocytes are provided by cellular connections extending to canaliculis.

The space in which osteocytes are found are named *lacuna*. *Lacunaes* are connected to each other and to the outer surface of bone via *canaliculi*. The nutrition of osteocytes are provided by cellular connections extending to canaliculis (10). These cellular connections are thought to play functional role in sensing new bone formation areas (14).

For many years, osteocytes are assumed as inactive bone cells embedded in bone matrix. Recently, osteocytes are known to regulate bone deposition and resorption by

receiving and responding to mechanical and hormonal stimulants. These cells coordinate osteoclastic and osteoblastic activity and contribute to bone homeostasis. Functional disorders in osteocytes have been associated with skeletal disturbances in which total bone mass decrease and risk of fracture increase.

2.1.1.4. Osteoclasts

Osteoclasts are giant, multi-nucleated phagocytic cells responsible of bone resorption. They rise from hematopoetic stem cells in bone marrow and are found in cavities on bone surface named *Howship lacunae.*

Basal portion of these cells contains 2-50 nuclei and various organelles. Vesicular region, located between the basal portion and ruffled border, contains enzymes functioning in resorption (15). Ruffled border, which consists of fingerlike processes similar to microvilluses, is formed by osteoclasts that are in close vicinity with the bone surface (16). This border increases the surface of cell membrane from which hydrochloric and proteolytic enzymes will be expressed (13). When osteoclasts migrate to resorption area, they attach to mineralized tissue surface with ruffled border and start to secrete enzymes extracellularly. Thus, mineralized tissue breakdown takes place.

Osteoclasts are characterized with expression of tartrate resistant acid phosphatase (TRAP), osteoprotegrin (OPG), cathepsin K. Among these, OPG blocks the interaction between nuclear factor kappa B (RANK) and RANK ligand (RANKL). Cathepsin K plays role in destruction of matrix proteins. RANKL that is expressed by osteoblasts stimulates osteoclastic differentiation, which indicates the importance of osteoblasts in osteoclastic differentiation.

Osteoclasts are affected by hormones expressed from parathyroid and thyroid glands.

2.1.2. Alveolar Bone

2.1.2.1. Structure of Alveolar Bone

Alveolar bone is the portion of the maxilla and mandible forming tooth sockets and supporting teeth. It is formed following eruption of tooth and resorbed after tooth loss.

Alveolar bone consists of three parts: outer cortical layer, compact bone lining the inner socket and cancellous bone between these two layers. The part of the alveolar bone that covers inside of the socket is called *bundle bone or alveolar bone proper*. Radiographically, this surface to where periodontal fibers attach is named *lamina dura.* Cancellous bone supports alveolar bone. Interdental septum is composed of cancellous bone surrounded by compact bone (10).

The morphology of the alveolar bone varies in terms of shape, size and axial inclinations of teeth. Cortical bone of the lower jaw is thicker compared to upper jaw. However, cortical bone in premolar and molar region shows increase (9).

Alveolar bone is mineralized connective tissue composed of organic matrix and water. Twenty three percent of the bone is composed of mineral contents, while 37% is the organic matrix, the majority of which is collagen, and the remaining 40% is water (19).

The socket wall reflects the responsiveness of alveolar bone to external forces. While osteoblasts and newly formed osteoid are located at the areas of tension, osteoclasts and bone resorption are seen at the pressure areas (20,21).

The junction of bundle bone and outer cortical layer is alveolar crest. In young adults, alveolar crest is located 0.75-1.49 mm apically to cementoenamel junction, whereas this distance increases with age to an average of 2.81 mm (27).

Bone marrow spaces, in which blood cells and osteogenic cells are present, are found in cancellous bone.

2.1.2.2. Remodeling

The necessity of bone resorption and deposition to be in equilibrium, in order for the bone to adapt mechanical loading and tensile strength, was defined by Frost in 1990 as ‘bone *remodeling*’ (32). *Remodeling*, is required to maintain the structural integrity of the skeleton and to achieve its own metabolic functions (calcium and phosporus reservoir) (33). Life-long deposition and resoption take place simultaneously, and thus total mass of the bone is maintained.

Remodeling cycle consists of three consecutive phases: activation, resorption and formation. The cycle initiates with activation of osteoblast lineage cells (osteoblast precursors, osteocytes). These cells transform and express RANKL. RANKL interacts with RANK, an osteclast precursor receptor. This interaction leads to differentiation of

hematopoetic cells into multi-nucleated osteoclasts. Osteoclasts start resorption via expression of hydrogen ions and enzymes, cathepsin K in particular. Osteoclastic resorption results in Howship lacunae in trabecular bone, Havers channels in cortical bone. Once the osteoclastic resorption is complete, reverse phase, in which single nucleated cells in bone surface is seen, takes place. These cells stimulate osteoblastic differentiation and migration, and prepare bone surface for new bone formation. In formation phase, resorption cavities are filled with osteoblasts. Bone matrix to be mineralized in the future is synthesized (33,34). Even though the process of migration of osteoblastic precursors are not fully covered, it has been reported that mesenchymal cells are transformed into osteoblastic lineage cells via expression of Runx2 gene (51).

The integrity of bone is controlled by hormones, bone marrow cells and other proteins expressed by bone cells. Function of bone is under systemic and local regulations (34). Systemically influencing molecules are parathyroid hormone (35), calcitonin, Vitamin D_3 (36), growth hormone (37,38), glucocorticoids (39), thyroid hormone (40), estrogen (41,42) and androgen (43). Responses to mechanical force changes, microfracture repair and maintanance of *remodeling* cycle is controlled locally (33).

Cytokines (IL-1, IL-2, IL-3, IL-6, IL-10, TNF-α) (45,67), prostaglandines (50,52), leucotriens, nitric oxide (33), colony stimulating factors (M-CSF, GM-CSF) (46), growth factors (TGF-*β,* IGF, FGF, PDGF) (38,44,47,48,49) are the local regulators of bone *remodeling.* Besides, it has been advocated that osteopontin (OPN), an extracellular matrix protein, regulates osteocyte function under tensile strength and plays crucial role in bone formation (75,76).

2.2 Orthodontic Tooth Movement

2.2.1. Orthodontic Tooth Movement Theories

2.2.1.1. Bioelectric Theory

When external forces are applied to long bones, the surface curvature of the bone changes in shape. Similarly, when teeth are subjected to orthodontic force, convex and concave surfaces occur as a result of flexing or bending of the alveolar bone, which has high elasticity (1). The resulting stress in the bone causes electrons to migrate and an electric current called ‘piezoelectric’ to occur. While negatively charged concave surfaces

stimulate osteoblastic activity, positively charged convex surfaces stimulate osteoclastic activity (2). Despite the fact that piezoelectric theory had been highly supported in 1960s and 70s, with better understanding of roles of growth factors, cytokines and other biochemical mediators in bone *remodeling,* and since piezoelectric current was also seen in dead bone cells, it has been clarified that this theory can not explain orthodontic tooth movement alone (4).

2.2.1.2. Pressure-Tension Theory

Orthodontic force applied to tooth crown moves the tooth inside the socket. Periodontal fibres at the direction which teeth are forced to move are compressed (*compression side*), and the fibres at the other side of the socket are stretched (*tension zone*). The circulation in the compression zone is destroyed and cellular breakdown occurs. On the other hand, vasodilation is seen at the tension zone. This change in blood flow in PDL leads to synthesis of crucial molecules as neurotransmitters, cytokines, growth hormones, coloni-stimulating factors and arachidonic acid metabolites (5). These molecules provide suitable environment for resorption in the compression zone and deposition in the tension zone. Thus, bone is resorbed in compression, and new bone is formed in the tension zone (6,7).

2.2.2. Tissue Reactions to Orthodontic Tooth Movement

Basically, there is no significant difference between tissue reactions observed in physiological tooth movement and the ones in orthodontic tooth movement (29). Since the tooth moves faster when the orthodontic force is applied, the tissue reactions seen as result are more significant and extensive (7). Orthodontic tooth movement is thought to be the result of site-specific remodelling in the absence of inflammation (22). Resorption and deposition mechanisms seen in skeletal functional adaptation are also valid for orthodontic tooth movement (53). It has been widely accepted that tensional forces stimulate osteoblastic cell proliferation while compressive forces excite osteoclastic activity (22).

Burstone categorized orthodontic tooth movement as follows: *initial* phase, *lag* phase and *post-lag* phase (3). *Initial* phase, seen in 24-48 hours after orthodontic force is applied, is characterized by immediate or rapid displacement of tooth in its socket. Tooth

displacement in PDL and bending of the alveolar bone result in spontaneous tooth movement. In this phase, the amount of tooth movement for light and heavy forces are approximately the same. The following phase, *lag* phase, lasts for 20-30 days and shows relatively little to no tooth displacement (99). This phase is the phase in which cellular components recruit to form tooth movement. Lag phase is shorter when light forces are applied while the phase is longer under heavy forces. Hyalinized tissue is removed after lag phase and orthodontic tooth movement occcurs afterwards (28).

Orthodontic tooth movement is related with resorption and deposition of tooth socket. Capillary vasodilation in PDL and bone deposition at the socket wall is observed in the tension region both under light and excessive forces. New bone islands are oriented parallel to the direction of periodontal fiber arrangement. In the compression zone, under light forces, osteoclasts located in Howship lacunae cause direct resorption of the alveolar bone. When under heavy forces, periodontal ligament is compressed at the compression zone, vasculature is compromised and cellular differentiation is inhibited. This leads to cellular and vascular breakdown followed by cell death. As a result, area termed 'hyalinization zone' appears. This is histologically cell-free zone which have glass-like appearance when viewed under light microscobe (4). It represents a sterile necrotic area, generally limited to 1 or 2 mm in diameter. Cells in hyalinization zone can not differentiate into osteoclasts and no resorption takes place in periodontal membrane. Thus, tooth movement stops (29). Following a delay of few days, cellular elements from undamaged periodontal ligament area migrate to hyalinized zone, and osteoclasts from neighbor bone marrow spaces start to remove bone adjacent to necrotic periodontal ligament area. This phenomenon is called 'indirect bone resorption', since the attack is initiating from beneath lamina dura rather than directly from PDL. Orthodontic tooth movement resumes after hyalinized tissue is completely removed by indirect bone resorption (28).

According to the studies in the literature, hyalinization in the pressure zones of PDL appears during initial phase of tooth movement, after only few hours of force application. However, Böhl et al. (331) found out that small hyalinized patches were present also in the later stages of tooth movement.

A systematic review revealed that clear relationship between force level, timing and extent of hyalinization could not be found. It was stated that magnitude of 5 cN also resulted in hyalinization and the timing of the event seemed to be independent of the force level (333). Interestingly, Tomizaku et al. (330) indicated that initially light and gradually

increasing force resulted in less hyalinization than a heavier initial force that increased to the same end force level.

The limiting factor of the rate of tooth movement ise bone resorption. Bone resorption rate is highly related with the number of osteoclasts. The access of osteoclasts to the bone in the direction of tooth movement is limited by pressure zones and necrotic areas in the PDL. Therefore, indirect bone resorption is required in the areas where the pressure is high and vascularity of PDL is jeopardized.

2.2.2.1. Biochemistry of Orthodontic Tooth Movement

Sensitivity of periodontal tissues to external forces renders orthodontic tooth movement possible (23,24). Mechanical loading via orthodontic force results in tension in PDL cells and extracellular matrix. This tension is responsible for the changes in blood flow and PDL vasculature. As a consequence, several key factors such as neurotransmitters, cytokines, growth factors, colony-stimulating factors and arachidonic acid metabolites are expressed.

In the early phase of tooth movement, acute inflammatory response is developed. This response is characterized with increase in vascular permeability, leukocyte migration and cytokine production leading to expression of prostoglandines and growth factors. Acute phase is followed by chronic phase, in which osteoblasts, fibroblasts and endethelium cells are proliferated and alveolar bone is gone under remodeling.

Cytokines in the gingival area during orthodontic tooth treatment provide information about local cellular metabolism, reflecting the status of periodontal health and bone remodeling (307). Cytokines are produced during activation of immune system cells and they function as intercellular signaling proteins. Orthodontic force results in vasodilation in periodontal capillaries and consequently, inflammatory cells move to the area and start to produce cytokines. Interleukin-1β (IL-1β), interleukin-6 (IL-6) and other inflammatory cytokines, being the first molecules to appear in orthodontic tooth movement, enable osteoclastic resorption (54). These proteins stimulate osteoclastic resorption by activating RANK-RANKL complex. It has been reported that IL-1β is expressed 12-24 hours after force application (72), whereas IL-6 is expressed 24 hours after mechanical loading (73). It also has been shown that expression of both of these cytokines reached the maximum level at day 3 and started to decrease afterwards (54). IL-8 has also been associated with bone remodeling in orthodontic patients. In a study

conducted with the gingival fluid sample, the level of this cytokine showed significant increase at the end of one month and it was stated that release of this cytokine would be possible in presence of sufficient amount of IL-1 (71).

TNF-α is proinflammatory cytokine that activates osteoclastic resorption. Changes in concentration of this molecule in PDL were noted following orthodontic force application. It was indicated that TNF-α is highly expressed, at pressure zone in particular (5,58,70,71).

In increasing RANKL level, compressive forces are more important compared to tensile forces. RANKL level shows increase in 24 hours following mechanical loading. Since presence of RANKL is detected in periodontal tissues in experimental tooth movement studies, it can be concluded that RANKL expression is regulated by inflammatory cytokines. In experimental researches, it was shown that compressive forces increase RANKL expression in periodontium by 16.7 times and decrease osteoprotegerin expression by 2.9 times (73, 74).

Chemokines, which are low molecular weight cytokines, were shown to increase after mechanical loading. It was stated that expression of chemokine CC Ligand 2 (CCL2) in periodontal ligament is increased after orthodontic force application. Decrease in osteoclastic and osteoblastic activity were also noted in the absence of CCL2 (56). Likewise, CCR5 was also found to suppress bone resorption in orthodontic tooth movement (55).

Matrix proteinases (MMP) contribute to remodeling by making destruction in extracellular matrix. Pressure in PDL had resulted in increase at mRNA levels of MMP-8 3 days after mechanical loading. This increase had disappeared after 2 weeks. Expression of this protein also displays similar manner in tension region (57). Apart from this, expression of MMP-2 is as follows: In the first hour, it increases significantly in the tension and reaches the basal level after 8 hours. Meanwhile, in the pressure zone, it reached the maximum level after 8 hours. The expression pattern of MMP-2 indicates that this molecule can be used as biological marker in active tooth movement (59).

One other molecule of importance in regulating of bone deposition and resorption is nitric oxide (NO). NO is a free radical with a short half-life. Expression of this molecule by osteoblasts and osteocytes has been proved under tensile forces (77). This molecule has been suggested to alter the RANKL / OPG balance towards bone formation (81). Nevertheless, IL-1 and TNF, which are associated with resorptive activity, have been reported to stimulate NO expression. There are studies pointing out that low-dose NO is

involved in IL-1 associated bone resorption while NO at high concentration inhibits osteoclastic activity (78,79). NO level in gingival crevicular fluid has been compared following orthodontic force application. In the pressure zone, NO levels have increased one hour following force application. On the other hand, delayed increase was seen in the tension zone after 3-4 days (80). The expression of this molecule also showed similar pattern in experimental animal studies on tooth movement (82,83).

Prostaglandines (PG), which are arachidonic acid metabolites, are synthesized shortly after wounding (61,62). They are expressed highly in inflammatory response due to cell wounding (64) and highly associated with bone resorption (101). Researches have shown that orthodontic force application enhances prostaglandine expression (5,68). As mechanical stress makes deformation in osteocytes, these cells were stated to express prostaglandines (63) and prostaglandines are known to initiate bone resorption via osteoclastic activation (66). PG concentration in gingival crevicular fluid is found to be significantly higher in both tension and pressure zones (65). It was indicated that PGE levels shows an increase in 24 hours at the pressure zone firstly, lowering to basal levels at the end of 7 days (66,102). Also, slowing down of orthodontic tooth movement by endomethacine (prostaglandine expression inhibitor) injection stresses role of this molecule in orthodontic tooth movement (69).

Osteocalcin, being the most specific biomarker in determining osteoblastic function, is non-collagenous matrix protein. It is synthesized by mature osteoblasts (83). Not only are there *in vitro* studies (84, 89) indicating that compressive forces do not affect the level of this molecule, but also *in vitro* (85,87,89) and *in vivo* studies (86,88) which demonstrate osteocalcin levels increase are present. Accumulation of this molecule particularly in tension zone points out the effect of osteocalcin in osteoblastic differentiation and bone apposition (71,90).

Transforming growth factor-beta 1 (TGF-β_1), which is effective in regulating regional bone remodeling, is one of the cytokines that can be expressed by osteoblasts, osteoclasts and fibroblasts (91). TGF-β_1 stimulates osteoblast production, extracellular matrix formation, and thus, bone apposition (97,99). It has been showed that TGF-β_1 is synthesized by osteoblasts under mechanical stress (94). It has also been stated that levels of this molecule increase during orthodontic tooth movement (92). Osteoclasts in compression zone and osteoblasts in tension zone have been stated to express TGF-β_1. This result clarifies contribution of this molecule in both soft and hard tissue remodeling (91). It has been advocated that release of this molecule is initiated 1 hour following

orthodontic force application in cats (93). When expression of this molecule in tension and pressure zones were evaluated, variable results were found. There is a research reporting that TGF-β_1 is released in higher levels in tension zone (96). However, in another study, TGF-β_1 levels which were higher compared to control side were found to be same in tension and pressure zones (71).

Vascular endothelial growth factor (VEGF) is a signaling molecule that takes critical part in angiogenesis (104) and functions in bone resorption and apposition (105,106). This molecule serves great importance in orthodontic tooth movement, particularly in periodontal remodeling (111). Orthodontic tooth movement researches have revealed that expression of VEGF in periodontal ligament was increased during tooth movement, and this increase was more significant in the pressure zone (108,109).

In rats, expression of VEGF was observed at day 1 and increased at day 7, at which indirect resorption became clear (104). However, it has been stated that VEGF release was also seen in tension zone (111).

One of the molecules of great importance taking part in tooth movement is macrophage colony stimulating factor (M-CSF). This molecule recruits osteoclasts and stimulates early osteoclastic differentiation (111). It was shown that periodontal ligament level of this molecule was increased during orthodontic tooth movement (110). Similarly, *in vitro* PDL cells were reported to express higher levels of M-CSF under compressive forces (113).

Orthodontic tooth movement is based on the principle of triggering of tissue remodeling by transitory, aseptic inflammatory response of inflammatory molecules. Hence, these inflammatory elements are required for orthodontic tooth movement (67, 71).

2.3. Acceleration of Orthodontic Tooth Movement

The long duration of orthodontic treatment is a compelling factor for the patients. As the duration of the treatment is prolonged, motivation and cooperation losses can be observed in addition to various complications (root resorption, periodontal problems, caries) (114).

Several factors including age, genetic factors, medicine intake (eg: NSAID, bisphosphonates), nutrition, systemic conditions (eg: pregnancy, diabetes, cancer, menopause) have been showed to influence the rate of orthodontic tooth movement (115,

116). These factors, alone or in combination, can affect remodelling activity of the bone, and thus the amount of tooth replacement (117).

Bone *turnover* rate dictates the extent of orthodontic tooth movement (114). When the bone turnover rate is high the tooth movement is accelerated while the tooth movement is slower as the turnover rate decreases.

Since bone to volume ratio of trabecular bone is higher than that of cortical bone, trabecular bone undergoes remodeling more actively (34). Deguchi et al. showed that the upper molars move faster than the lower molars in dogs. They suggested that the reason for this is the bone resisting tooth movement is basically in trabecular structure and the osteoclasts migrating to the area are attacking from bilateral trabecular structure (118).

Bone remodeling decreases as the age increases. Reitan investigated tooth surrounding structures histologically and stated that adult periodontal ligament contains fewer cells compared to young individuals (119,120). As a result of biochemical analysis of gingival crevicular fluid, it was reported that mediators in young individuals responds to mechanical stimulus quicker than adults and thus, initial tooth movement is started without lag phase in young individuals (125). These results support that tooth movement in young people is faster than adults (117,122). Furthermore, the decrease in RANKL/OPG ratio due to age was stated to be related with tooth movement in the early phase (123). Similar results were obtained from rat studies (121,124). Osteoclasts were seen after 2 weeks following force application in young rats while in adult rats, this duration was 4 weeks (121).

Osteoclastic activity is the limiting factor in orthodontic tooth movement (53). The principle of accelerating tooth movement is based on stimulating osteoclastic acitivity and thus increasing bone turnover. Interventions in this field can be categorized as chemical approaches, physicomechanical approaches, gene stimulation, surgical approaches.

2.3.1. Non-Surgical Methods in Accelerating Orthodontic Tooth Movement

2.3.1.1. Chemical Applications

Prostaglandins

Prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) are inflammatory mediators stimulating bone resorption. PGE_2, in particular, stimulates bone resorption via increasing osteoclast number directly (67). The effects of PGs on orthodontic tooth movement has been shown in several researches. Yamasaki (68, 127) has been one of the first researchers investigating local PG injection on rats and monkeys. The tooth receiving local PG injection is reported to move two times faster than the control side. Besides, he also mentioned that osteoclasts were present and bone resorption was evident at the injection side. Kale et al. (136) concluded that after 9 days of experimental period, PGE_2 injection increased osteoclastic activity and accelerated orthodontic tooth movement. Likewise, Leiker et al. (128) stated that PGE_2 injection accelerated orthodontic tooth movement in rats and single or multiple injections made no statistically significant difference in terms of rate of tooth movement. Moreover, there are studies indicating that when applied together with Ca, PGE_2 application also stabilizes root resorption in addition to accelerating tooth movement (129). The effect of chemically produced PGE_2 on canine retraction was investigated in a *split-mouth* clinical case study in which first premolars were extracted. In this study, it was stated that tooth movement in retraction side was 1.6 times faster compared to control side (130). In a histopathologic study in which the effects of PGE_2, that is applied in different methods, on tooth movement and bone metabolism was investigated, it was reported that local and intraligamentous injection of PGE_2 increased tooth movement amount together with osteoblast and osteoclast numbers. Moreover, intraligamentous injection was found to be more effective compared to local application (138).

One other way of indirectly influencing PGE_2 synthesis is via diet rich in omega-3 fatty acids. The bony lipid samples of rats which were fed with this kind of diet for 5 weeks revealed decreased arachidonic acid and PGE_2 levels (134). In rats fed with diet rich in omega-3 fatty acids, incisor movement was slower as well as buccal movement of upper 1^{st} molars (135).

Lee (131) examined the effects of local and systemic administration of PGE_1 on bone resorption in rats. He found that bone resorption was more significant in compression zone in systemic administration. He also mentioned that due to rapid destruction of PGE_1 in lungs and side effects as inflamation and local irritation, local application of PGE_1 would be limited. In order to accelerate tooth movement without these side effects, PGE_1 analogue was investigated. When this molecule was given orally to rats, orthodontic tooh movement was found to be accelerated and at low doses, root resorption was stated to be at minimum levels (132).

The effect of indomethacin, which is a prostaglandin synthesis inhibitor, on orthodontic tooth movement was investigated. As a result of their study in which elastic seperators were placed between the indomethacin-injected molars, Yamasaki et al. (68) concluded that osteoclasts were not present in the interradicular septum. Similarly, Mohammed et al. (137) pointed out decreased tooth movement in rats which were administered indomethacin systemically. Tuncay and Chumbley (69) showed that oral administration of indomethacin ended up with decelaration of tooth movement in cats.

Non-Steroid Anti-Inflammatory Drugs (NSAIDs)

NSAIDs act via inhibiting cyclooxygenase enzyme (COX) activity. Cyclooxygenase, the key enzyme for conversion of arachidonic acid into prostaglandins, exists in two isoforms (COX-1 and COX- 2). COX-1 has been suggested to synthesize prostaglandins that are responsible from integrity of the gastric mucosa, while COX-2 takes part in synthesis of prostaglandin responsible from pain.

NSAIDs have analgesic, anti-pyretic and anti-inflammatory effects (142). In terms of chemical content, they can ben categorized as follows: salysilates (eg: aspirin), profens (eg: ibuprofen, flurbiprofen), arylalcenoic acids (eg: diclofenac, indomethacin), fenamic acids, COX-2 inhibitors (eg: rofecocsib).

Researches support the fact that NSAIDs slow down orthodontic tooth movement. They are highly related with increase in activity of matrix metaloproteinases (MMP9 and MMP2) and collagenase enzyme and decrease in procollagen synthesis, which is required for remodeling (143).

Acetyl salysilic acid (ASA), also known as aspirin, inhibits expression of prostaglandin via inhibiting both types of COX enzymes (141) and hence decreases bone resorption (143). Wong et al. (144) claimed that daily ASA administration under light

forces (8 cN) did not inhibit the movement of lateral tooth in animals. However, under 35 cN force, significant inhibition of lateral tooth movement was seen in rats which were administered ASA two times a day (145). Also, in another research, local injection of ASA was stated to inhibit tooth movement in rats. (146).

Chumbley and Tuncay (69) reported that orthodontic tooth movement was very slow in patients who were administered aspirin for a long time. Moreover, they also noted that significant increase in tooth movement was seen as the intake of this medicine was stopped. Nevertheless, when the first dose is taken before force application, short term administration (<3 days) of NSAID was reported to not to prolong the treatment time (53).

Similar results were obtained from studies conducted on diclofenac (147) and ibuprofen (145, 150). These molecules were claimed to inhibit orthodontic tooth movement. However, flurbiprofen was reported to have no adverse affect on tooth movement (151).

The fact that factors synthesized by COX-1 enzyme takes role in bone remodeling oriented the researchers to study COX-2 inhibitors. In a study in which the effects of diclofenac and rofecoxib molecules, cox inhibitors, were compared; it was noted that both molecules inhibited tooth movement without any statistically significant difference between the groups (147). On the other hand, another study revealed that specific COX-2 inhibitors had no effect on orthodontic tooth movement (148).

Leukotrienes

Leukotrienes, a product of arachidonic acid metabolized by lypooxygenase enzyme, stimulate bone resorption (149). Therefore, these were thought to have role in orthodontic treatment. In an experimental tooth movement study, 60 cN of force was applied together with leukotriene inhibitor in rats and the results revealed that the tooth movement was inhibited significantly (137). Close coil springs were placed between the rat incisors and molars in another study. It was suggested that osteoclastic differentiation and bone resorption due to force application was inhibited in rats which were administered leukotriene inhibitor orally (152).

Interleukins

Interleukins are crucial cytokines in bone remodeling. IL-1 stimulates osteoclastic activity by its receptors on osteoclasts (5) and is the earliest identified bone resorption biomarker (54).

Soluble cytokine receptors play key role in understanding the functions of cytokines. These receptors neutralize cytokine activity by binding to ligands to which cytokines were supposed to bind. It was shown that local application of soluble receptors led to significant decrease in osteoclastic activity and bone loss. Similarly, orthodontic movement was stated to be slower when soluble IL-1 receptor was administered systemically to rats (159-61). Also local injection of IL-12, which inhibits tumour necrosis factor (TNF)-α-mediated osteoclast formation, was reported to inhibit both mechanical tooth movement and root resorption during orthodontic tooth movement in mice (110). Also, it was reported that the amount of tooth movement was reduced when IL-4, inhibitor of osteoclastic activity, was injected (158).

1,25-Dihydroxycalciferol

1,25-Dihydroxycalciferol (1,25 DHCC) is the biologically active isoform of vitamin D. As a result of decrease in serum calcium levels, parathyroid hormone expression is stimulated and thus, in order to maintain calcium homeostasis, renal phosphate excretion, calcium reuptake and 1,25 DHCC expression is increased (153).

Since this molecule is known to stimulate osteoclastic differentiation and activity (154, 155), it was also shown that bone remineralization was stimulated by 1,25 DHCC (156).

The effect of 1,25 DHCC on orthodontic movement in rats was investigated in several studies. In one of them, 1,25 DHCC was injected palatally to upper first molars of rats in different concentrations and molar tooth was moved buccally. It was noted that tooth movement increased in a dose dependent manner (153). In another research, spring was placed between the incisors and PGE and 1,25 DHCC injections were made locally close to gingival area. It was suggested that both molecules increased the amount of tooth movement, 1,25 DHCC being more effective in regulation of bone turnover (136). A study based on canine retraction in cats revealed similar results (157).

In a thesis study, effect of local administration of 1,25 DHCC on orthodontic tooth movement was evaluated in rats. It was stated that this agent results in increase in osteoclastic activity and bone resorption (162).

Osteocalcin

Osteocalcin (OC) is the most abundant non-collagenous, extracellular matrix protein of bone. Since this molecule has high affinity to calcium and hydroxyapatite, osteocalcin functions as negative regulator in bone formation.

Kobasyashi et al. (166) investigated local application of OC on orthodontic tooth movement. They placed elastic separators between upper 1st and 2 nd molars. Researchers indicated that number of osteoclasts increased in the compression zone in the alveolar bone. They emphasized the role of OC on recruitment of osteoclasts and thus increase in amount of tooth movement. Likewise, Hashimoto and Kobayashi (167), in their experiment of 10 days, suggested that local application of OC accelerated upper 1st molar mesialization in rats.

Nitric Oxide

The role of nitric oxide (NO) in bone formation and destruction has prompted investigators to question the effect of this molecule on orthodontic tooth movement. Hayashi et al. noted that buccal movement of the rat molar was significantly reduced when NO inhibiting agent was locally administered (163). Akın et al. (164) examined the effects of agents stimulating and inhibiting NO synthesis. Agents were applied in addition to orthodontic force. Authors found out that Howship lacunae, number of osteoclasts and orthodontic tooth movement were significantly higher in group, in which NO synthesis was stimulated, compared to other one. Similarly, Shirazi et al. (165) reported that tooth movement is increased in the group in which NO synthesis was stimulated, and reduced in the group in which NO synthesis was inhibited. Authors also underlined that root resorption was less in the group in which NO synthesis was stimulated, compared to control group.

Corticosteroids

Corticosteroids are class of steroid hormones produced in adrenal cortex. They function in inflammatory and immune reactions, response to stress, carbohydrate metabolism, protein catabolism and regulation of blood electrolyte levels.

Researches point out that corticosteroids act on bone tissue by inhibiting osteoblastic activity and reducing general bone formation. They are also related with increased bone resorption (171). Osteoporosis was reported in long term use of corticosteroids (169, 170).

Corticosteroids accelerate tooth movement. However, they decrease the stability of the movement since new bone formation is reduced (172). A study revealed that in this type of osteoporosis-initiated rabbits, active tooth movement is higher than that of the control group, but stability is less and new bone formation is not observed (171).

Ong et al. (173) examined rate and stabilization orthodontic tooth movement in rabbits with osteoporosis, which were given corticosteroids. As a result, they claimed that skeletal resorption is increased, bone apposition is suppressed and hence tooth movement is accelerated.

Kalia et al. (174) evaluated short and long term administration of corticosteorids and rat tooth movement. In one of the experiment groups, tooth movement was initiated following 7 weeks of prednisolone injection while in the other group, metil prednisolone was administrated with the tooth movement simultaneously. Researchers stated that tooth movement was accelerated in both groups, being more in the chronic group.

Parathyroid Hormone

The main function of parathyroid hormone (PTH), which is secreted from parathyroid gland, is to increase the amount of calcium in the blood. Continuos elevation of PTH results in bone resorption as intermittent increase in small amounts leads to anabolic effect (175).

The first research concerning the effect of PTH on orthodontic tooth movement was conducted on rats in 1970s and it was stated that this hormone accelerates tooth movement by increasing bone turnover. Other studies on rats revealed that exogenous PTH application increased tooth movement amount in dose-dependent mannor. This

increase was observed in continuous local (177) and systemic (176) infusions of PTH. Besides, orthodontic tooth movement was seen to be faster when PTH was slowly released, and thus the residence time of PTH in the injected area is increased, by using this hormone together with a gel medium (177).

The indirect role of PTH on orthodontic tooth movement can be clarified by researches on low calcium diets. It was claimed that PTH levels are increased in experimental animals fed with low calcium diet (178,179).

Thyroxine

Thyroxine (T_4) is one of the two hormones secreted from thyroid gland. It plays important role on cell activity and metabolism when the inactive form of T_4 is activated (T_3). Furthermore, T_4 indirectly contributes to bone turnover by acting on intestinal calcium absorption. T_4 administration is related with increase in bone remodeling and resorption activity, decrease in bone density (172).

In order to clarify the effect of thyroxine on orthodontic tooth movement of rats, 25 cN was applied to upper first molars of rats for 21 days. Thyroxine was added to drinking water of the animals and 4 weeks following significant increase in tooth movement was observed (114). Another study noted that intraperitonal administration of thyroxine at different doses resulted in dose dependent increase in mesial movement of molars (186).

Proton Pump Inhibitors

Proton pump inhibitors (PPIs) inhibit the proton pumps of gastric acid producing cells. They are used safely in the treatment of gastrointestinal disesases. Decrease in calcium absorption and osteoporosis can be observed at high dosages.

Shirazi et al. (294) evaluated the effects of pantoprazole, proton pump inhibitor, on orthodontic tooth movement on 72 rats. Mesial movement of the first molar was compared at the end of 2 weeks and the result was as follows: When compared to control group, there was significant increase in tooth movement of the groups which received propranazol 4 and 5 weeks prior orthodontic appliance placement.

Tromboxane

When arachidonic acid is metabolized with cyclooxygenase, tromboxanes and prostaglandines are formed. Tromboxanes function as vasoconstrictors and facilitate platelet aggregation. Even though they show an increase in inflammatory situations as deep periodontal pockets, no relationship was established between tromboxanes and periodontal bone loss (187).

Yamasaki et al. (68) studied the effects of imidazole (specific trombaxane A_2 synthesis inhibitor) on orthodontic tooth movement of rat molars using elastomeric separator. They reported decrease in number of osteoclasts in the interdental septum of the group receiving. Also, Gürton et al. (139) noted significant increase in the movement of rat incisors, which were administered trombaxane analogue in every 12 hours locally.

Relaxin Hormone

Relaxin hormone (Rln), which facilitates birth by expanding pubic ligaments during labor, is known to act on physiological functions such as angiogenesis, bone turnover in both females and males (180). This hormone is found in periodontal ligament and cranial sutures and was reported to have effect on differentiation and activation of osteoclasts (182,184). Relaxin takes part in soft tissue remodeling rather than hard tissue.

Yang et al. (185) examined Rln release from rat ovaries during orthodontic tooth movement. Rats were sacrified after 6, 48 and 144 hours following orthodontic force application. They concluded that level of this hormone significantly increased in rat ovaries.

More recently, randomized clinical trial was carried out to evaluate the effect of relaxin on tooth movement and stability. 50 μg of relaxin or placebo was injected to 40 subjects for 8 weeks. Tooth movement was measured weekly on polyvinyl siloxane impressions which were scanned digitally. When relaxin and placebo groups were compared, the results revealed no difference in tooth movement amount over 8 weeks of treatment or relapse at 4 weeks post-treatment (183).

Liu et al. (181) evaluated the effect of Rln on rat tooth movement. They administered Rln either subcutaneously or by mini-osmotic pump. In both experiment groups, the amount of tooth movement increased on day 3 compared to control group. At

the end of 14 days, the authors observed that tooth movement amount was still higher than that of control group. They advocated that Rln might have accelerated tooth movement at the early phase of orthodontic treatment and also added that mini-osmotic pump method, by which the blood concentration of the hormone is maintained, is superior to subcutaneous application.

However, Madan et al. (180) showed that administrating Rln by mini pump did not accelerate orthodontic tooth movement; but it might have reduced organization and mechanical strength of periodontal fibers and thus, increase tooth mobility. In this research, in vitro experiment was also conducted to test the strength of the periodontal ligament. As a result, the influence of Rln on orthodontic tooth movement has not been clarified yet.

Gene Transfer

Osteoclastogenesis is regulated primarily by two cytokines: RANKL and MSCF. RANKL, which is cell membrane protein, is found in the surfaces of osteoblasts and stromal cells while its receptor RANK is found in osteoclast lineage cells. RANKL plays crucial role in regulating bone resorption balance between RANK and OPG, which inhibits expression of RANKL.

Rapid absorption of chemical agents in the circulation and their daily administration requirement have prompted researchers to accelerate tooth movement by gene transfer.

Kanzaki et al. (188) evaluated tooth movement on upper first molar of 6 week-old rats by applying force palatally via fixed orthodontic appliance. Authors have reported that osteoclastogenesis and tooth movement amount was decreased in the group, which received local OPG gene periodically. Same authors applied RANKL gene palatally of upper 1st molars and observed that osteoclastogenesis and the amount of tooth movement increased significantly (189). Parallel to these studies, Dunn et al. (190) evaluated molar mesialization under two different doses of OPG gene. They applied NiTi springs between incisors and molars. OPG gene that was injected mesially to upper first molars was found to decrese molar mesialization amount and number of osteoclasts. At the same time, they noted that amount of incisor retraction changed dose dependently.

2.3.1.2. Mechanical Stimulation

Since chemical approaches have side effects, the focus on acceleration of tooth movement was drawn towards non-invasive vibrational stimuli.

Nishumira et al. (195) applied buccal force, together with vibrational stimuli (60Hz), to upper first molar of Wistar rats. At the end of 21 days, histological analysis of the samples revealed increase in number of fibroblasts and osteoclasts of the experiment group. Moreover, amount of tooth movement of the experiment group was found to be higher than that of control group. This influence was suggested to be result of RANKL activation. However, Miles et al. (192) found that 20 min/day application of mechanical vibration at a frequency of 111 Hz did not accelerate tooth movement.

Kalajzic et al. (193) have shown the inhibition effect of mechanical vibration on orthodontic tooth movement on 26 Sprague-Dawley rats. Mechanical vibration (30 Hz frequency, 40 grf) was applied to experimental group two times per week for 10 minutes each. At the end of the study, decrease in the amount of tooth movement was reported. Researchers also underlined the destructive effect of this force on periodontal ligament. They suggested that force magnitude of this amount (40grf) might have caused such a result.

Yadav et al. (194) studied the effect of low frequency mechanical vibration (LFMV) on the rate of tooth movement on 64 mice. Loading protocols for each animal consisted of 15 minutes of LFMV at 1 cN of force at a frequency of 5, 10, or 20 Hz. LFMV was applied to the maxillary right first molar at 3-day intervals. After 14 days of experimental period, no significant difference was observed between the experiment groups in terms of rate and amount of tooth movement. However, it was noted that maximum tooth movement was at low frewuency (10 Hz), Even not statistically significant, authors reported that the activity and number of osteoclasts decreased.

Leethanakul et al. (198) evaluated the effect of vibrational stimuli on expression of IL-1B on 15 individuals via canine distalization. Vibrational force (125 Hz) was applied by electric tooth brush, 15 minutes/day for 2 months and ginigival crevicular fluid samples were taken. At the end of the experiment, it was stated that tooth movement amount was increased as well as the expression of IL-1B in experiment group.

Woodhouse et al. (196) used an intraoral vibrational appliance called *Acceledent* on 81 patients who were indicated with extraction of upper 1st premolars. Patients were

only evaluated at leveling and aligning phase of the treatment. It was reported that this appliance did not accelerate tooth movement and had no effect of shortening the treatment time. However, the effect of *Acceledent* on rate of orthodontic tooth movement and root resorption was investigated in a case report at Texas University. The rate of tooth movement was found to be higher in the experiment group than that of the control group. When Acceledent application for 6 months was evaluated, root resorption was stated to be in clinically acceptable borders (197).

Another kind of vibrational stimuli is low frequency ultrasound. Ultrasonic pulse waves (300-100 mW/cm^2) contribute to soft and hard tissue recovery by making biochemical changes on cellular and molecular basis. Xue et al. (191) examined molar mesialization on 48 Wistar rats. Ultrasonic vibration (20 min/day, $30mW/cm^2$) was applied to all experimental groups (1,3,5,7 and 14-day groups). Authors reported that orthodontic tooth movement accelerated after the fifth day.

2.3.1.3. Electromagnetic Stimulation

Exogenous electric current can cause changes in skin, cartilage, tendon and bone tissue just as it can influence cellular behaviour. In *vitro* studies revealed that electrical stimuli could initiate expression of epidermal and vascular growth factors (199-201).

Davidovitch et al. (202) evaluated the effect of electric current on orthodontic tooth movement on 15 female cats. At the end of the experiment, they noted that the teeth receiving 15 μA moved significantly more than the control group. In the study, they pointed out that there was an increase in the number of the osteoclasts, bone apposition and the densities of cyclic adenosine monophosphate (cAMP) and cyclic guanasine monophosphate (cGMP) in the periodontal ligament in the tension zone.

Kim et al. (203) investigated the effect of exogenous electric current on human orthodontic tooth movement. They made canine retraction on 7 patients. 20 μA of current was applied to the experimental group for 4 weeks, 5 hours per day. In the experiment group, canine teeth moved 30% more than the control group. Also, amount of tooth movement was reported to decrease after first two weeks.

Spadari et al. (204) studied the effect of low intensity electric current on tissue reorganization during orthodontic treatment in 2016. 10 μA of current was applied to 32 Wistar rats for 5 minutes per day. At the end of the study, it was stated that the electric

current caused an increase in the number of fibroblastsi osteoclast and blood vessels, and regulated the release of TGF-β1, VEFG and FGF.

2.3.1.4. Laser Biostimulation

The biostimulative effect of the laser has been known for years. The tissue changes are related to wavelength. Low energy level is beneficial since the temperature of the treated tissue does not exceed normal body temperature (210). Low-density laser therapy (LDLT) stimulates fibroblast proliferation, collagen synthesis and bone rejeneration. Furthermore, it increases osteoblastic and osteoclastic activity (205-209).

The close relationship of orthodontic tooth movement with bone remodeling invited researchers to investigate the effects of LDLT on orthodontic tooth movement. Kawasaki and Shimizu (211), evaluated the effect of Ga-Al-As diode laser on the rate of tooth movement by mesializing rat molars. Orthodontic tooth movement was found to be 1.3 times faster on the experimental group treated with laser (9 min/day, for 12 days). Afterwards, Yamaguchi (212) and Fujita (213) revealed that LDLT increased orthodontic tooth movement by stimulating RANK/RANKL and M-SCF. Another study pointed out that Ga-Al-As diode laser increased the levels of MMP-9, cathepsin K and alfa(v) beta (3) integrin, which play role in osteoclastogenesis (214).

Cruz et al. (215) were the first researchers to investigate the effects of LDLT on orthodontic tooth movement in humans. In their *split-mouth* study on 11 patients, it was noted that Ga-Al-As diode laser (100 sec/day, for 8 days) increased orthodontic tooth movement significantly on the experiment side. Youssef et al. (216) conducted similar clinical study in 2008. In their canine retraction study on 15 patients, they evaluated the effects of Ga-Al-As diode laser on orthodontic tooth movement and pain reaction during the treatment period. LDLT was applied at intervals of 3,7 and 14 days. At the end of the treatment it was stated that the rate of canine tooth movement on the experiment side was significantly increased and pain levels were noted to be lower on the experiment side.

On the other hand, researchers that advocate LDLT has no effect on orthodontic tooth movement are present too. Kim et al. (219) investigated the effect of LDLT on periodontal fibronectin and type I collagen turnover in 30 rats. Ga-Al-As diode laser (10 sec/day, for 7 days) was applied to animals to which elastic separator was placed between maxillary incisors. Researchers indicated that LDLT has no effect on orthodontic tooth

movement but contributed to reorganization of periodontal connective tissue. Seifi et al. (217) evaluated the effects of two types of low-laser wavelengths (630 nm and 850 nm) on orthodontic tooth movement in rabbits. LDLT was applied to 18 albino male rabbits for 9 days and the animals were sacrified on day 16. The findings of the study implied that the amounts of orthodontic tooth movement, after low-level laser therapy, were diminished. Altan et al. (218) conducted a similar study on 15 patients with premolar extraction. Ga-Al-As diode laser was applied on 1st, 2nd, 3rd and 7th days of canine retraction. Gingival crevicular fluid samples were taken 1 and 48 hours following the force application. As a result of immunologic and stone cast analysis, no significant difference was found in terms of orthodontic tooth movement amount and PGE-2 and IL-1 levels between experiment and control groups.

2.3.1.5. Platelet Rich Plasma

Platelets, which constitutes approximately 6% of the peripheral blood, play crucial role in both soft and hard tissue healing due to their growth factors (220). The platelet concentration of platelet rich plasma (PRP), which is prepared by autogenous centrifugation from the patient's blood, is 5-10 times higher than that of the normal blood. It is stated that PRP may have the potential to accelerate wound healing and increase regeneration by increasing the number of platelets in the wound area and thus, increasing the local concentration of growth factors (221). PRP has been applied in dentistry for its capability of enhancing osseointegration of a dental implant and augmentation of alveolar bone height in maxillary sinus lift (220).

In a case report, Liou (220) indicated that submucosal injection of PRP increased the amount of orthodontic tooth movement by 1.7 times in the leveling and aligning phase. The author claimed that the effects of single dose injection of PRP can last for 5-6 months and emphasized that maximum acceleration of tooth movement is between 2nd and 4th months. Besides, it was also noted that, in order to accelerate tooth movement more than two folds, the optimal PRP fold should be between 9.5 to 12.5 folds.

Güleç et al. (222) investigated the effects of local injection of PRP on rat orthodontic tooth movement. In this *split-mouth* study, 40grF was applied between upper incisors and upper 1st molars. It was stated that on the experiment side, alveolar bone

density, which was calculated by *Cavelieri* method, was decreased from day 3 to day 21 and returned to baseline by day 60.

2.3.1.6. Platelet Rich Fibrin

Platelet rich fibrin (PRF), first established in 2001 by Choukroun et al. (223), is a second-generation platelet concentrate used in soft and hard tissue healing. The concept of PRF is based on centrifugation of the blood that is withdrawn to a anticoagulant-free tube. In few minutes, a fibrin layer is formed in the middle of the tube as a result of coagulation cascade. After careful isolation, the fibrin is ready to use. By this application, platelets and their released cytokines can be kept in the fibrin. The target of this process is to provide expression of the cytokines for longer period (224). Recent studies show that critical growth factors are released from PRF for 7 to 28 days (225, 226). Furthermore, ease in application and not requiring biochemical handling makes it superior to PRF (224).

In a clinical study in 2016, the effects of using L-PRF in periodontally accelerated orthodontics (PAOO) on post-operative pain, inflammation and post-orthodontic stability was investigated. 11 orthodontic patients received corticotomy and bone graft together with L-PRF. At the end of two years, accelerated healing of flap was observed in every patient. The severity of pain was noted as mild to moderate, with no severe pain. The researchers stated that average treatment time was 9,3 months and orthodontic stability was remained for 2 years after the surgical procedure (227).

2.3.1.7. Growth Factors

Epidermal growth factor (EGF) is takes place in stimulation of proliferation of epithelial and mesenchymal cells as well as synthesis of macro molecules and bone resorption. Also, it is accepted as the natural regulator of dental eruption (228,229).

Saddi et al. (230) evaluated the effects of local administration of EGF on recruitment of osteoclasts during mechanical force in rats. They examined upper first molar mesialization on 32 rats. The injected EGF solution to furcation point of the first molars. As a result, they found out that EGF enhanced osteoclast recruitment and thus increased bone resorption and accelerated orthodontic tooth movement. Similarly,

another rat study reveals that EGF promoted RANKL expression and stimulated osteoclastogenesis and tooth movement (231).

Another critical growth factor is vascular endothelial growth factor (VEGF). VEGF, being a key molecule in angiogenesis, also stimulates osteoclastogenesis (232).

Kaku et al. (233) investigated the effect of local administration of VEGF on osteoclastic differentiation on rat tooth movement. Helical springs were placed between the incisors and VEGF was administered to buccal side of the incisors. It was pointed out that number of osteoclasts significantly increased at the pressure side of the PDL. Kohno et al. (234) manifested that local administration of VEGF significantly accelerated orthodontic tooth movement. Parallel with these findings, same researchers indicated that the rate of orthodontic tooth movement and osteoclastic differentiation decreased in the mice which were given anti-VEGF antibody (235).

2.3.2. Surgical Approaches

2.3.2.1. Distraction Osteogenesis

The main principle of distraction osteogenesis is mechanically stretching the recovering bone at the osteotomy or corticotomy site by means of distraction device and thus forming the new bone.

Starting off from this finding, Liou et al. (283) compared orthodontic tooth movement with distraction osteogenesis. The claimed that stretching of periodontal fibres at the tension zone (distraction) and formation of new bone at this site (osteogenesis) is basically similar. In 1998, they performed canine distalization by periodontal ligament distraction on 15 patients with 1st premolar extraction. Following extractions, researchers made grooves in the buccal and lingual walls of interseptal bone by burs. Afterwards, tooth bone distraction device, which is activated 0.5-1 mm/day, is placed in the mouth. They reported that canine tooth moved 6.5 mm to the extraction site and posterior anchorage loss is minimal.

Following this research, Kişnişçi et al. (284) conducted clinical study on 11 patients who required bilateral first premolar extraction and canine distalization by 'dentoalveolar distraction'. Teeth extractions are made following osteotomies around canine tooth and buccal cortical bone is removed carefully to obtain mobile transport

segment. Distractor is placed between 1st molar and canine tooth. Distraction was initiated at the day of replacement with the rate of 0.8 mm/day. The device is removed from the mouth when sufficient amount of distalization was achieved. At the end of the study, authors reported that there was no anchorage loss, root resorption or loss of vitality.

As there are other clinical trials supporting accelerated canine distalization by dentoalveolar distraction (286-88, 290), are there also successful trials with periodontal ligament distraction (285).

In a study comparing these two approaches, it was found that, even though it has more complicated surgical approach, dentoalveolar distraction is more successful compared to periodontal distraction in terms of root resorption, distraction duration and tooth vitality (289).

In some studies, activation is started right after surgical intervention (283-85) while in other studies activation is initiated following latency period (286-88, 290). The common point of these studies is this: With fixed orthodontic treatment mechanics, distalization is achieved after 4-5 months while this period is significantly shortened by distraction osteogenesis and anchorage loss is minimal. The underlying reason for this is that canine teeth, which has less resistance to move, can distalize easily while anchor teeth are not moving within 3 weeks, which is the time required for the hyalinised tissue to be removed. Authors suggest that the intended tooth movement should be obtained in this 3-week period in order to avoid anchorage loss.

2.3.2.2. Corticotomy

Corticotomy is first described in 1892 as ‘cortical incisions of the alveolar bone to reposition the teeth and stabilize them in their new position’ and attracted the real attention in 1959 by case report series that presented different treatment approaches (237-39).

Kole advocated that the main factor resisting tooth movement is the cortical bone and he suggested that the integrity of the cortical bone should be destroyed to accelerate tooth movement. For this purpose, without destroying the structural integrity of the trabecular bone, he reflected full-thickness flap and made vertical bone incisions in the interproximal cortical bone extending tooth apex. He combined these incisions via

subapical horizontal incisions including trabecular bone. In this technique, in which corticotomy and osteotomy are combined together, the researcher reported that teeth are moving as '*bony blocks'* instead of moving alone, and major teeth movement is achieved in 6-12 weeks. By this method, he treated diastema cases, open and deep bite malocclusions by means of correcting incisor inclination (237-239).

Based on Kole's technique, Düker evaluated the effects of accelerated tooth movement on marginal periodontium and vitality of teeth (240). As a result of his study on Beagle dogs, he stated that dental pulp and PDL is not harmed with corticotomy and the closest corticotomy incisions should be made 2mm apical to the marginal bone.

Later, in a case report published in 1978, Generson et al. (241) showed that orthodontic correction took place in a short period of time with alveolar decortication without subapical osteotomy on 2 patients. In the following years, Suya (242) conducted a clinical study as 'corticotomy-assisted orthodontic treatment' on 395 patients. Researcher advocated that the bony blocks, in which the teeth are embedded, should be in connection with the neighbour bony blocks by trabecular bone. Based on this consideration, different from Kole's technique, subapical horizontal osteotomy cut was replaced with horizontal corticotomy cut. Researcher noted that the average treatment time was between 6-12 months and also underlined the fact that orthodontic treatment should be finished 3-4 months after corticotomy and prior to fusion of bony blocks, which were separated with corticotomy. Moreover, he specified that by this technique, the treatment period was less painful, produced less root resorption and exhibited less relapse.

In the advancing years, Wilcko brothers contributed new dimension to orthodontics. In two case reports published in 2001, corticotomy-assisted orthodontic treatment was accompanied with alveolar bone grafting in patients with severe crowding. After reflecting mucoperiosteal flaps buccally and lingually, selective alveolar decortication was made on the bone walls. The design of the decortication was as follows: Interproximal vertical corticotomy cuts were connected with horizontal cuts and numerous perforations were made on the cortical bone. Neither the cuts nor the perforations ruined the integrity of the trabecular bone. Researchers stated that the modification of the design was due to increase marrow penetration rather than creating bony blocks. Flap was closed primarily after grafting following decortication. Orthodontic activations were made in every 2 weeks after surgery. At first, this technique was named as '*accelerated osteogenic orthodontics(AAO)'*, which later transformed into

'*periodontally accelerated osteogenic orthodontics, (PAOO*)'. Approximately after 6.5 months of orthodontic treatment time, researchers specified that orthodontic tooth movement was accelerated and the buccal bone thickness was increased (248-50).

Wilcko brothers suggested that orthodontic tooth movement was related with transitory remineralization-demineralization process of the alveolar bone rather than 'bone block' movement. Researchers advocated that the factor accelerating tooth movement is based on *regional acceleratory phenomenon (RAP),* which was first described by orthopaedist Harold Frost. Frost (126) implied that surgical wounding of the bone resulted in significant *remodelling* in soft and hard tissues adjacent to site of injury. He attributed all of these physiological events that were characterized with increased bone turnover and local osteopenia during healing as *regional acceleratory phenomenon (RAP).* Frost implied that *RAP* process progressed as follows: initial rapid osteoclastic activity which was followed by decrease in the bone density; afterwards rapid osteoblastic activity and initiation of *remodelling* process. He advocated that healing of this kind was 2-10 times faster compared to physiological healing. It was stated that orthodontic force alone could stimulate mild *RAP* reaction and *RAP* reached to maximum levels with corticotomy-assisted tooth movement (236).

The idea of 'reflecting a mucoperiosteal flap alone can arouse RAP response' was investigated in a study by Yaffe et al. (243). Researchers reflected buccal or both buccal and lingual flaps in rat mandibles of 60 Wistars rats. As a result of radiologic and histological analysis, it was observed that RAP response was stimulated 10 days after surgical injury and the most of the resorptive activity took place in the first week reaching to maximum level in the 3rd week. Bone resorption was more significant in the group which received both buccal and lingual flaps. They also reported that the first phase of RAP was characterized with increase in cortical bone porosity due to enhanced osteoclastic activity. Furthermore, they claimed that in humans, a response like this can start few days after the surgery, reach to maximum levels in 1-2 months and cease in 6-24 months. As a matter of fact, Aboul-Ela et al. (252) conducted a study supporting this hypothesis. Researchers evaluated corticotomy-assisted canine retraction in *split-mouth* study on 13 adult patients with 1st premolar extractions. When compared to control side, the orthodontic tooth movement on the corticotomy side was accelerated 2-fold in the first two months, 1.6-fold on the 3rd month and 1.06-fold on the 4th month.

Lee et al. (245) designed a study on 30 rats to clarify whether the teeth move by means of distraction osteogenesis or RAP response. They categorized the animals into 5 groups as follows: corticotomy alone, corticotomy-assisted tooth movement, osteotomy alone, osteotomy-assisted tooth movement and tooth movement alone. Researcher concluded that alveolar response varied according to types of bone cut. As a result of micro-CT analysis after 21 days; while RAP response, which is characterized with regional bone loss, was seen in the corticotomy group, similar pattern to distraction osteogenesis imitating fracture healing was encountered in the osteotomy group.

In order to reveal healing phases of RAP, Schilling et al. (314) performed drill hole defects (1.2 mm in diameter), which were extending from one cortical side to the other through marrow cavity, at tibia of 48 rats. The results revealed that, at the initial stage, woven bone extending from periosteal region to trabecular zone is formed. The woven bone reaches to its maximum thickness at day 7. It is required to maintain mechanical stability after wounding. Starting from day 7, as woven bone in the cortical bone goes under remodelling to transform into lamellar bone, woven bone in the trabecular bone goes under resorption. This reveals transitory osteopenia. Trabecular bone will undergo remodelling after the structural integrity of the new cortical bone is obtained.

Sebaun et. al. (236) evaluated alveolar and periodontal response to selective alveolar decortication in terms of time and proximity to site of surgery. Since orthodontic tooth movement alone can stimulate RAP response, the study was designed to not to include tooth movement. Full thickness triangular flaps were reflected adjacent to upper 1st molars of 36 rats. 10 decortication dots were made at the buccal and palatal aspects of the cortical bone (5 marks at each side) using 0.2 mm half-round burs. Afterwards, flaps were closed with sutures. At the end of 3 weeks, significantly less mineralized trabecular bone surface, increased periodontal surface area, as well as increased number of osteoclasts and increased apposition width in the lamina dura was exhibited in the surgery group. Catabolic and anabolic activities accelerated by 3 times, mineralized trabecular bone decreased by 2-fold and PDL surface increased by 2-fold. Moreover, tissue turnover rate reached to constant level at the end of 11 weeks. Besides, it was stated that the effect of surgical injury was localized to the area immediately adjacent to the decortication injury.

Iino et. al. (244) evaluated the effects of corticotomy on orthodontic tooth movement and alveolar bone on 12 beagle dogs. In their *split-mouth* study, at the experiment side, full thickness flaps were reflected to reveal buccal and lingual cortical bones at the mandibular 3rd premolar region and corticotomy was made afterwards. Tooth movement was performed under 0.5 N of force on 3rd premolars using sentalloy close-coil springs. Results revealed that, when compared with the control side, orthodontic tooth movement amount on the experiment side was significantly higher and tooth movement rate was 2-5 times higher than that of the control side. At the same time, while PDL hyalinization on the corticotomy side was observed 1 week after surgical operation, on the control side, hyalinised tissue was observed during 4 weeks after surgery. It was also noted that no root resorption existed on the corticotomy side as significant root resorption, particularly at the root surfaces adjacent to hyalinization zone, was observed in the control group following surgery at 4th and 8th weeks.

In 2010, Teixeira et. al. (282) conducted a study from the hypothesis that shallow perforations of the maxillary cortical bone can increase expression of inflammatory cytokines and bone remodelling, and can accelerate orthodontic tooth movement. For this reason, 48 rats were divided into 4 groups: orthodontic force alone, orthodontic force plus soft tissue flap, orthodontic force flus soft tissue flap plus 3 small perforations and control group. 50 cN was applied to upper 1st molars of the experiment group using Sentalloy close-coil springs. Soft tissue flap was reflected around 1st molars and sealed with tissue adhesive. 3 shallow perforations (0.25 mm in diameter, depth of 0.25 mm) located 5 mm mesial to the 1st molar were performed by means of a round bur and slow handpiece. The animals were killed at day 28 and maxillae were dissected. Micro CT and immunological analysis revealed that tooth movement amount was highest in the perforation group and also when compared to other groups, cytokine release was significantly increased in this group.

In 2011, Baloul et. al. (251) investigated the effects of selective alveolar decortication (SAD) on orthodontic tooth movement on 114 rats. Animals were categorized into 3 groups as SAD only, tooth movement only and SAD supported tooth movement. Full thickness flaps, revealing buccal and lingual cortical bones of upper 1st molars, were reflected and 25 g of force was applied to first molars by Sentalloy springs. 5 decortication marks (width and depth of 0.25 mm) were made on each cortical surface. The flap was primarily sutured. At the end of 7 days, orthodontic tooth movement amount

was found to increase significantly in the SAD group These findings were accompanied by reduced bone volume and mineral content in the experimental group. Furthermore, RNA markers of major osteoclastic cells and regulators (M-CSF, RANKL, OPG, calcitonin receptor, tartrate resistant acid phosphatase 5b) indicating osteoclastogenesis were shown to increase. Researchers concluded that alveolar decortication increases orthodontic tooth movement by means of influencing bone resorption and deposition in the early stages of tooth movement.

In 2017, Kurohama et al. (297) investigated the effect of bone volume cut removed during corticotomy, on orthodontic tooth movement, root resorption and alveolar bone resorption. Two different orthodontic forces (10 g and 25 g) were applied for upper 1st molar mesialization and two different time periods were designed. As subgroup of these groups, volumes of the bone cut by corticotomy were 0.1, 1.0, and 1.7mm^3 in the 25g-force groups, and 1.0 and 1.7mm^3 in the 10g-force groups. As a result of their study, they concluded that the volume of the alveolar bone cut during corticotomy had no effect tooth movement or root resorption; however, alveolar bone loss might be increased following tooth movement, manifesting as reduced height of the alveolar bone.

Many clinical and experimental researches in the literature on corticotomy-assisted tooth movement supports that this method accelerates orthodontic tooth movement (246-47, 253-61, 308-09).

Even though the effectiveness of surgical techniques requiring mucoperiosteal flap reflection is proved, invasive procedures are hardly accepted by the patients due to post-surgical complications and discomfort. Therefore, more conservative techniques have been developed in the recent years that do not require flap reflection (263, 275).

In 2006, Park et al. (275) introduced corticision technique based on accelerating tooth movement by making cuts through the gingiva with surgical blade and hammer transmucosally.

Kim et al. (263) proposed that clinically, it is impossible to adjust the magnitude of the force to avoid hyalinization and therefore, *lag* phase could be shortened by accelerating resorption of the hyalinized tissue. They claimed that this could be achieved by creating suitable environment for tissue remodelling, which is enabled by RAP. Thus, they evaluated the effect of corticision on alveolar bone remodeling by means of canine distalization on 16 cats. The magnitude of force used to distalize canines was 100 g. In

corticision technique, they performed transmucosal corticotomy cuts on interproximal cortex. The cut was started 2 mm apical to the interdental papillae and extended 1 mm beyond the mucogingival junction. The surgical blade was positioned with inclination of 45-60° to the long axis of the tooth. The surgical blade was inserted gradually from interradicular gingiva into the trabecular bone by malleting. The cuts were made on the mesiobuccal, distobuccal and distopalatinal alveolar cortex of the canine teeth. At the end of day 28, researchers concluded that corticision accelerated alveolar remodelling by stimulating anabolic and catabolic activities, and hyalinezed tissue was removed more rapidly in the experimental group. However, Murphy et al. (276) investigated the effects of corticision on orthodontic tooth movement and alveolar response on rats under two different force magnitudes. In the experiment group, corticision was made only on the mesiopalatal cortex. MicroCT and histomorphometrical analysis revealed that, regardless of the force magnitude, there was no significant difference between the groups in terms of orthodontic tooth movement. As bone volume fraction showed decrease in the corticision and heavy-force-only groups; in light-force-only group, this value was found to be significantly lower than the other groups.

In a study investigating the effects of light (LF) and heavy forces (HF) with corticotomy on tooth movement rate, alveolar bone response, and root resorption in a rat model. Maxillary molars were mesially moved by closed-coil spring delivering either 10 g (light force) or 50 g (heavy force). For the corticotomy procedure, following reflection of full-thickness flaps on both cortical plates, two decortication marks were made on both the buccal and palatal sides by using 0.5-mm round carbide bur. Depth of the decortication marks were adjusted to be half of the bur diameter. Tooth movement and alveolar bone response were evaluated on days 7, 14, 21 and 28. It was stated that the OTM amount in the HF group was significantly greater than in the LF group on days 7 and 14. However, no significant difference was found between the two groups at days 21 and 28. In terms of BV/TV ratio, the LF and the HF groups presented no significant difference at all studied time points. Similarly, no significant difference was detected between the groups regarding root resorption during the experiment period (332).

Even though the corticision technique significantly shortens the duration of the operation, it does not offer the benefits of bone grafting as Wilcko technique. Afterwards, a method allowing bone or soft tissue grafting and that has higher patient acceptance was developed. In 2009, Dibart et al. (264) performed corticision with ultrasonic waves and

introduced the piezocision technique. In this technique, following orthodontic force application, vertical cuts are performed as far as possible from the interdental papillae on the buccal side. The cuts were designed as to pass periosteum and contact with the cortical bone. Going through these cuts, corticotomy cuts with 3 mm depth were made with ultrasonic device (piezotome). By tunnel approach, soft and hard tissue grafting can be performed in the cases requiring bone augmentation. In this technique, the need for suturing is diminished except for the cases requiring graft stabilization. Authors demonstrated that moderate crowding can be solved in 17 weeks. Nhane et al. (265) showed that this technique caused no periodontal harm. Keser and Dibart (266) revealed that piezocision accelerated orthodontic treatment with clear aligners and advocated that it could be beneficial in getting more esthetic outcomes. Kim et al. (271) evaluated orthodontic tooth movement by perforating the cortical bone (*piezopuncture*) directly from the gingiva without making any cuts with piezotome. The study was conducted on 10 beagle dogs and 100 g of force was used to perform orthodontic tooth movement. At the end of 6 weeks, they observed that orthodontic tooth movement is accelerated and bone *turnover* has increased.

Other piezoelectric studies also support that orthodontic treatment can be accelerated with less trauma and periodontal integrity can be preserved by this technique (267-70, 272-74).

Since the laser application is both less traumatic and accelerates soft tissue healing by reducing the risk of bleeding and bacterial infection in the application area, Seifi et al. (277) investigated the effect of applying flapless corticotomy with the help of Er, Cr: YSGG laser in 2012 on the effect of orthodontic tooth movement on experimental animals. In their studies on 8 New Zealand rabbits, they assessed the amount of lower 1st premolar mesialization. On the experimental side, in addition to 75 g of orthodontic force, the laser-assisted bone corticotomy was made under air-water cooling without contacting the bone. As a result of the 21-day trial, the amount of orthodontic tooth movement on the experimental side was found to be significantly higher than the control side. Similarly, in Erdem's (279) study on rats in 2012, the effect of Er: YAG laser decortication, with transmucosal approach, on orthodontic tooth movement was investigated. Sentalloy close coil springs, with continuous force of 25 cN, were used for molar mesialization on 28 Sprague-Dawley rats. In the experimental animals, decortication dots were made on three surfaces of the upper 1st molar teeth. On stone cast models, the distance between upper

1st and 2nd molar teeth were measured both prior to orthodontic force application and on day 7 and day 14. In addition, alveolar bone volume and total bone volume between 1st and 2nd molar teeth were counted. As a result of clinical and histomorphometrical evaluations, the orthodontic tooth movement amount, alveolar bone volume and total bone volume in experimental group was higher than that of the control group. The author suggested that transmucosal ER: YAG laser decortication could enhance bone remodelling.

In a clinical study in 2014, canine retraction was evaluated in 15 patients indicated with upper 1st premolar extractions. In the experiment group, flapless decortication dots by ER: YAG laser were performed both on mesial and distal side of the retracted canine. At the end of 6 weeks, data from stone cast models revealed that the amount of orthodontic tooth movement in the experiment group was 2- fold greater than that of the control group (278).

Alikhani et al., who showed that micro-osteoperforations (MOP) accelerated orthodontic tooth movement in previous animal studies, carried out another study to examine the effect of this application on humans in 2013.

In their study of 20 patients with upper 1st premolar extraction, they investigated the effect of micro-osteoperforations, performed transmucosally with Propel device, a minimally invasive procedure, on the amount of canine distalization and release of inflammatory markers. They designed the study as follows: In the control group, only canine distalization was made while on the experiment group, as *a split-mouth* design, one side received only canine distalization and the contralateral side received MOPs in addition to canine distalization. Alginate impressions were taken from the patients 6 months following teeth extraction prior to canine distalization. In cases where distalization of the canines were performed by applying 100 gr-force, three flapless micro-osteoperforations were made by a single-use Propel device on the distal side of the canines before the distalization. Alginate impression were taken again after 4 weeks and orthodontic tooth movement was evaluated. Also, in order to evaluate inflammatory response, gingival crevicular fluid samples were also taken from the patients prior to orthodontic treatment and before canine distalization. The amount of canine distalization measured on the stone casts revealed that distalization amount on the MOP side was 2,3 times greater than that of the contralateral side and control side. Also, inflammatory markers as cytokines and chemokines were found to be increase significantly on the MOP

side. Researchers concluded that transmucosal MOP application is a safe and effective method in accelerating orthodontic tooth movement and this approach could shorten the treatment duration by 62% (280).

Swapp et al. (313) conducted a study on 7 foxhounds evaluating the effects of cortical bone damage, which was performed by bone-awl, on the alveolar bone surrounding the moving tooth. The authors suggested that microfractures cause more bone destruction and osteocyte death than lesions incurred by drilling and greater amounts of bone damage are related with greater tooth movement. Furthermore, without going beyond the cortical bone, they wanted to limit the insults to the buccal and lingual cortical plates. For this purpose, 60 microfractures (small perforations) were made with bone awl, without periosteal flap, before initiating tooth movement. *Split-mout*h design was performed on 7 animals to evaluate protraction of the mandibular third molars for 56 days under 200 gr-force. MicroCT and histological analysis were performed to assess bone morphology and modelling. Radiographic and intraoral measurements were made to evaluate orthodontic tooth movement. At the end of the study, tooth movement assessments revealed no statistically significant difference between experimental and control sides. Buccal and lingual cortical bone volume fractions were significantly less on the experimental side than the control side. Mesial medullary bone volume showed no significant difference between sides. Buccal and lingual cortical bone densities on the experimental side were also significantly less whereas the density of the mesial bone was not. Also, experimental side showed significant buccal and lingual cortical bone modelling, particularly evident at the injury sites, but the control side demonstrated almost no modelling. The authors claimed that the reason of no acceleration of tooth movement could be attributed to maintenance of periosteum and not extending the effects of RAP to medullary bone. They also suggested that bone damage caused by microfractures, corticotomies can initiate remodelling but the effects are relatively limited compared with generalized damage caused by removing periosteal blood supply.

Cheung et al. (312) investigated the effectiveness of mini-implant-facilitated MOPs in inducing accelerated tooth movement and evaluated the potential risk for root resorption. In their *split-mouth* study on 6 male Sprague-Dawley rats, 5 MOPs were placed mesial and palatal to the maxillary first molar tooth. MOPs were created by inserting the mini-implant (1.2 mm width) into a depth of 1 mm using slow-speed implant driver. Molar mesial movement was performed with NiTi closed coil spring applying 25

gr-force for 21 days. The results revealed that the tooth movement on the MOP side exhibited 1.86-fold increase compared to control side. Bone mineral density was decreased in the experimental side. Also, increased bone loss characterised with shortened interradicular trabecular bone heights were also noted. Additionally, the osteoclast number surrounding the first molars was 44% on the MOP side compared to control side. Further MicroCT analysis revealed that all 5 roots of maxillary first molars showed no statistically significant difference between the sides. The authors concluded that MOPs, performed by mini-implants, can effectively accelerate tooth movement in rats.

In an animal study in 2016, the effect of flapless MOP and corticision on orthodontic tooth movement in rats was investigated. 45 Sprague Dawley rats were categorized into 3 groups: orthodontic force only group, orthodontic force with MOP group and orthodontic force with corticision group. Upper 1st molar mesialization was made with Sentalloy springs under 50 gr-force. Corticision was made by gently malleting the surgical blade until the contact with the cancellous bone was provided. 3 shallow MOPs were made with slow handpiece by using burs with 0.25 mm depth and width. Corticision and MOPs were made 5mm mesial to upper 1st molars in both groups. Micro-CT analysis were made to evaluate bone volume to total volume ratio. The amount of mesialization is assessed by weekly measurements of the distance between upper incisor and molars via digital calliper in anesthetised animals. At the end of 6-week period, the results demonstrated that difference in surgical methods made no significant difference between the groups, but, starting from the 2nd week, the tooth movement amount in the experiment groups was significantly greater than that of the control groups. Bone volume to total volume ratio was significantly reduced at the end of 6 weeks in all groups, more in the experimental groups (281).

In 2017, Librizzi et al. (310) compared the effects of corticotomy and flapless corticision, on the rate of orthodontic tooth movement in a rat model and evaluated the changes in the alveolus. For this purpose, 60 male rats were used and orthodontic tooth movement was made under 10-15 gr-force for 21 days. The corticotomy procedure consisted of reflecting a full-thickness flap and making 3 shallow perforations, each 0.25 mm wide and deep, using slow-speed hand piece, 5 mm mesial to the first molar. The bur was fully immersed into the bone during drilling. Corticision was performed transmucosally by using reinforced surgical blade. The blade was inserted gradually

penetrating the overlying gingiva, cortical bone and cancellous bone to approximately 1 mm. In the corticision + flap group additionally flap was elevated. Orthodontic tooth movement was evaluated by maxillary impressions on day 0, 7, 14, 21 and dental die stone models were fabricated. The results manifested that although the mesial molar movement amount showed incremental pattern for all groups with significant difference between 7,14 and 21 days; no significant differences were found between groups at any of the time points. Also, no significant difference was found in terms of BVF between different experimental groups. Moreover, osteoclast activity, numbers and surface area were not significantly different between groups.

At the same year, Peron et al. (311) compared the tissue responses to corticotomy- and corticision-assisted tooth movement in 90 rats. The animals were divided into 3 groups: tooth movement only (control group), tooth movement surgically assisted by corticotomy and tooth movement surgically assisted by corticision. In the corticotomy group, following reflection of the buccal and lingual flap, three shallow perforations were performed on the vestibular bone and two perforations were created on the palatal bone aspect. Perforations were made using ¼ -mm spherical carbide bur with low-speed handpiece. The corticision was made both on the lingual and vestibular bone plates, mesial and distal to the upper molar that was being moved. With inclination of 45-60 degrees to the long axis of the molar, a surgical scalpel was gradually advanced through the gums, cortical bone and cancellous bone. Following both surgical procedures, contact with medullary bone was confirmed by periodontal probe. Mesial molar movement was achieved with nickel titanium closed coil spring under 30 gr of force for 3, 14 and 28 days. The results of the histological analysis revealed that greater number of osteoclasts were observed in the corticotomy group compared to control group on day 3; however, the results were similar in the corticision and corticotomy groups. Also, lower percentage of immature collagen fibres were seen in the experimental groups compared to control group. However, no significant differences were found in terms of tooth movement rate between the groups at the time intervals. The researchers concluded that increased bone resorption occurred in the early stages of the tooth movement and the surgical procedures performed had no effect on tooth movement rate. They suggested that additional activations of the surgical procedure might have been required.

2.4. Stereological Investigation Method and Cavalieri Principle

Stereology, which is originally a Greek methodology, enables us to reach 3-dimensional information of objects by measurements made on its 2-D microscopic images. (98).

Systematic random sampling, which means giving equal opportunity to every point of a working structure, is important in quantitative *in-vivo* studies. The underlying example is intended to be valid for all structures or communities to be interpreted and to represent that structure or community correctly (279).

Stereological probes are geometric questions concerning the structure of interest and desired data. These can be 1, 2, or 3 dimensional according to the dimensional properties of the parameter of interest. Appropriate samples made with appropriate probes allow us to have information about the 3D situation in the space as well as the actual parameter of interest (76).

The Cavalieri method is based on the calculation of the volumes of three-dimensional objects that do not have a regular geometric shape by dividing them into parallel slices. With this method, the structure to calculate the volume is first divided into slices and the surface area of each slice is found and multiplied by the slice thickness to calculate the volume of the slice concerned. Finally, the volumes of the slices are summed together by the aggregate of interest (279).

By using a dot grid ruler, which is a transparent acetate with equal space of dots printed on the images, it is possible to obtain reliable results for the calculation of the cross-sectional surface areas in the images in a short period of time. In addition to this, some semi-automatic machines or special image analysis systems can be used (76).

3. MATERIALS AND METHOD

3.1 Materials

Ethical approval for this research was obtained from Yeditepe University Ethical Committee of Experiment Animal Care and Use (date: 11.01.2016, no: 500). 72 male Sprague-Dawley rats (311.94 ± 31.97 g, 16 weeks old) were obtained and acclimatized in Yeditepe University Laboratory Animal Science Center. Histological investigations were performed in Department of Histology and Embryology in the same university.

3.1.1. Materials Used In The Experimental Tooth Movement Phase

- Sterile injector (2 cc)
- Ketasol ® (Richter Pharma AG, Wels, Austria)
- Rompun® (Bayer Helayh Care LLC., Cansas, USA)
- Vynyl polysiloxane impression material (Express XT Quick, 3M ESPE AG, Seefeld, Germany)
- Sentalloy close-coil springs applying 50 gr-force (Dentsply GAC, New York, USA)
- 0.8 mm width stainless steel ligature wire (American Orthodontics, Wisconsin. USA)
- Low-speed handpiece (NSK, Kanuma, Japan)
- 0.8 mm diameter round bur (Bosphorous, İstanbul, Turkey)
- Dynamometer (Correx Tension Gauge, Haag-Streit International, Koeniz, Switzerland)
- Acid etching (37% phosphoric acid, Ivoclar Vivadent, Liechtenstein)
- Bonding agent (Transbond XT primer, 3M Unitek, Monrovia, CA, USA)
- Transbond Xt Light Cure Adhesive (3M Unitek Monrovia, CA, USA)
- Flowable composite (Filtek Z350XT, 3M ESPE AG, Seefeld, Germany)
- Digital caliper with a sensitivity of 0.01 mm (Unbranded, China)
- Dental stone cast material (Unibase-300, Dentona AG, Dortmund, Germany)

3.1.2. Materials Used in Histological Investigation

- 10% neutral formaldehyde (Merck & Co., ABD)
- %85 formic acid (Tekkim Laboratory Chemicals, Tuzla, İstanbul)
- Tri-sodium citrate (Tekkim Laboratory Chemicals, Tuzla, İstanbul)
- Tricrom-Masson (Sigma-Aldrich INC., St. Louis, MO, USA)
- Acid phosphatase, Leukocyte (TRAP) Kit (Sigma-Aldrich INC., St. Louis, MO, USA)

3.2. Method

72 male Sprague Dawley rats with a body weight of 265 to 400 grams were used for the experiment. The rats were divided into the following groups: control (orthodontic force only), 1D (force plus single decortication) and 3D (force plus three decortication) groups. Observations were made on days 7, 14 and 21.

Following weight measurement, the rats went under general anaesthesia with %10 Ketasol® (60 mg/kg/im, including 100 mg/ml ketamine hydrochloride, Richter Pharma AG, Wels, Austria) and %2 Rompun® (5 mg/kg/im, including 23,32 mg/ml xylazine, Bayer HealthCare LLC., Kansas, USA).

The mouth of the rats was opened with two separate pieces of gauze bandage passed over the upper and lower incisors and the edges of the bandages were secured onto the working table. Following this, the initial maxillary impressions were taken from the animals by vinyl polysiloxane impression material (Express XT Quick, 3M ESPE AG, Seefeld, Germany).

Afterwards, retention grooves on the upper incisors and molars were prepared by high-speed handpiece using round bur. Due to continuous eruption of the rat incisors, retentive grooves on the upper incisors were prepared as gingivally as possible. Following this, one edge of the 0.08 mm stainless steel wire was passed through interproximal region beneath the contact points of upper 1^{st} and 2^{nd} molars and the other edge is passed through the retentive ring of the 50 g Sentalloy close coil spring. The wire was ligated to encircle the first molar.

The activation of the spring was provided by elongating the spring and fixing it to the retention grooves by 0.08 mm stainless steel wire. The amount of force was standardized with dynamometer (Haag-Streit Diagnostics, Berne, Switzerland) For additional retention, following acid-etching and primer application, both retention grooves on the incisors and molars were closed with flowable composite.

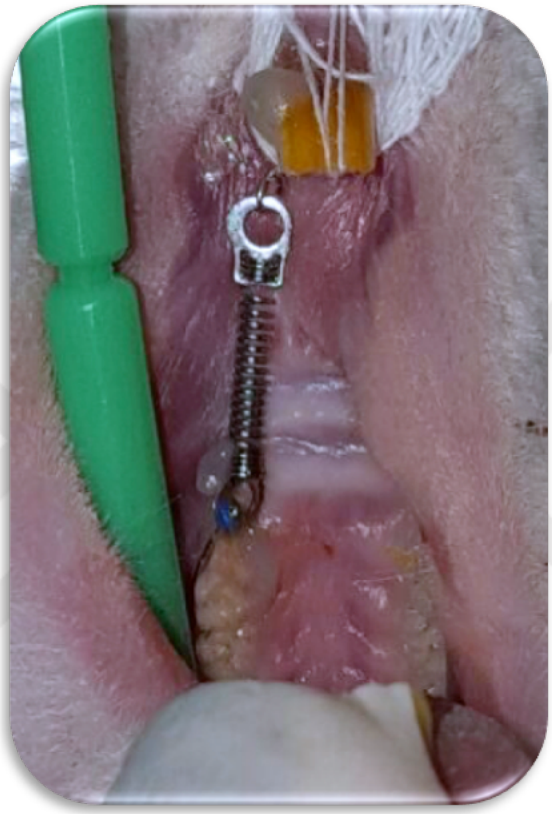

Fig. 2.1. Intraoral picture of Sentalloy close-coil spring placed between incisor and 1st molar of a rat

In the experimental group animals, shallow perforations were made either only on the mesial (single decortication group) or on the mesial, palatal and buccal sides (3 decortication group). Decortication process was performed using 0.8 mm diameter round burs with transmucosal approach under sterile water irrigation.

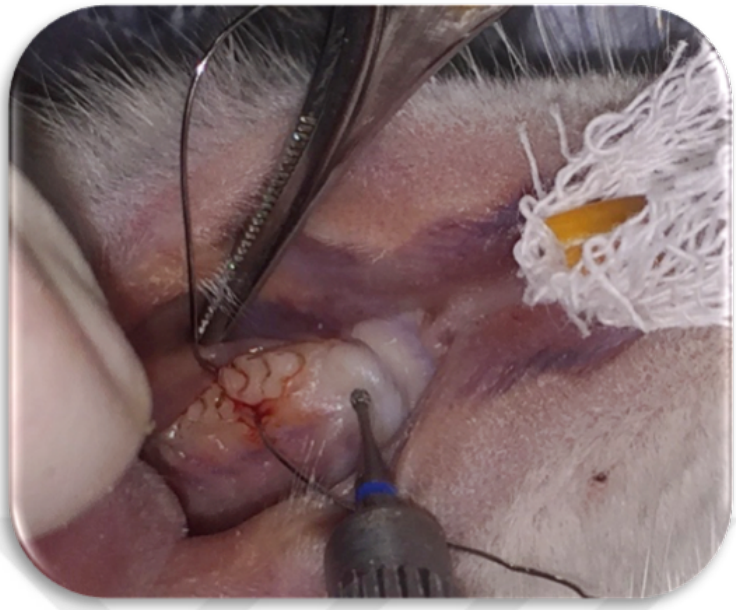

Fig. 2.2. Application of transmucosal decortication with slow-speed hand piece

Each of the animals were placed in a 60 x 40 x 40 cm cage without bedding materials, and then were acclimatized for 1 week before the experiments. They were maintained at a controlled temperature (22 ± 2°C), with 12 hr light/dark periods having free access to water and a commercial diet. To check that the appliances were in place, the animals were observed every other day and were monitored for weight follow-up.

The animals in the subgroups of both control and experimental groups were sacrificed by overdose anaesthesia after 7, 14 and 21 days from the beginning of the experiment according to the groups they belonged. The final maxillary impressions were taken following the removal of the Sentalloy spring and ligature wires.

3.2.1. Histological and Histomorphometrical Evaluation

After soft tissue dissection, maxillae were separated from the skull by means of surgical scissors. Tissue samples were fixed in 10% neutral formaldehyde in 0.1 mol of phosphate buffered saline solution (pH 5.74). Samples were decalcified with Morse solution (10% sodium citrate and 22.5 % formic acid) for 4 to 5 weeks. After sufficient decalcification was obtained, maxillae were divided in 2 hemimaxillar halves along the midpalatal suture by surgical blade and the halves in which orthodontic tooth movement

were practiced were embedded in paraffin. Paraffin blocks were sectioned longitudinally (10 mm thick) parallel to the long axis of each tooth, from mesial to distal aspects. Later, every ninth tissue sample section was selected through a set of consecutive sections. Choosing the first section was done in a systematic random manner. Sampled sections were stained with Masson's trichrome technique.

In order to assess osteoclastic activity, only one section from each animal showing the most root and pulp structure was chosen to stain with TRAP (tartrate-resistant acid phosphatase). First, sections were dewaxed, rehydrated, and incubated in TRAP reagent; which was prepared according to Bancroft (327) for 1 hour at 37 C. They were then counterstained with 2% methyl green. A section incubated in substrate-free medium was regarded as the TRAP control. Osteoclasts were determined as multinuclear TRAP-positive cells on the mesial and distal sides of the mesial and distal roots of upper first molar, respectively. Images of the first molars were captured at 20-times magnification (DM 4000 B: Leica). Histomorphometrical analyses were carried out using Stereo Investigator (Version 11.0; MicroBrightField). Multinuclear TRAP + positive cells on the mesial and distal side of the upper 1st molar were counted as osteoclasts.

Histologically stained specimens were investigated in stereological work-station; which consisted of computer-controlled motorized microscope stage (Bioprecision, Howtrone, NY, USA), CCD Digital camera (Optronics Microfire 1600x1200P, Goleta, CA, USA), mikrokator (Heidenhein, Traunreut, Germany), display card (ATI FireGL Advance Micro Device, Camberly, England) and light microscope (Leica DM 4000B, Wetzlar, Germany). Volumetric measurements were made using Leica C Plan objective (magnification of X20). In order to analyse alveolar bone volume, Cavalieri estimator of Stereoinvestigator 11.0 (Microbrightfield, Williston, VT, ABD) software was used.

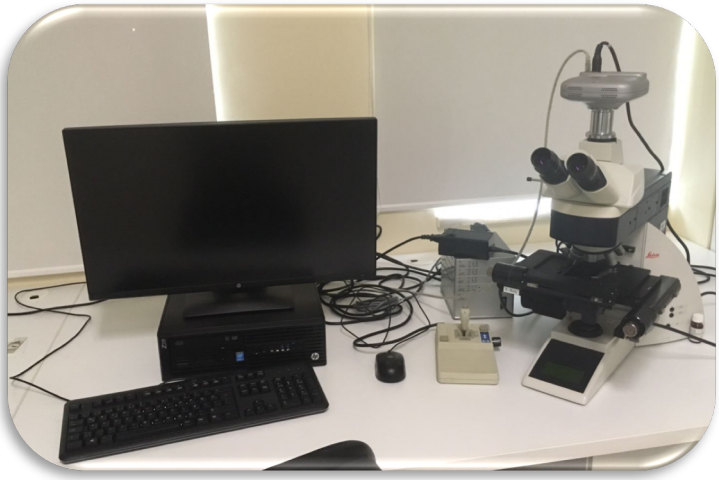

Fig 2.3. Stereological work-station

Alveolar bone volume was determined using Cavalieri principle of previously mentioned stereological work-station. The bone area found in between the upper first and second molars, which is cervically limited by the connective tissue, apically by apical foramen and mesiodistally by the cements of 1st and 2nd molars, was investigated. This area is buccopalatinally restricted by the first visible sign of root until the last visible sign root. The bone tissue in this boundary was defined as alveolar bone volume, whereas total soft and hard tissue in this area was defined as total volume. In order to estimate volume of each section, the maximum efficiency was obtained by using a point counting grid (d=50 μm). The representative areas per point (a/p) were 2500 μm^2 for each point. After applying the point counting grid to the sampled sections in a systematic random fashion, the numbers of points hitting alveolar bone in the sample were counted. Results were used for the estimation of alveolar bone volumes using the following formula:

$$\text{volume alveolar bone} = t\ (\mu m) \times a/p\ (\mu m^2) \times \sum p$$

where, t is the mean section thickness; a/p represents the area of each point on the point counting grid; and ∑p is the total number of points hitting the sectioned area. The alveolar bone volume located between two teeth was calculated by means of multiplying section sampling fraction by the total volume of sampled sections of each rat hemimaxillae. The efficiency of sampling and convenient point density for volume estimation were checked by estimation of coefficient of error (325) and coefficient of

variation as previously described (324). The alveolar bone volume/ total volume ratio (BV/TV), which is expressed as the percentage of the tissue volume consisting of alveolar bone over total hard and soft tissue volume (alveolar bone, periodontal ligament, connective tissue and bone marrow spaces) (326), was determined at 20-times magnification. BV/TV results enable the researcher to interpret bone density in the investigated area. The borders of the tissue area that were examined are shown in Figure 2.4. All measurements were made by the same investigator, who was blinded to the treatment groups and time points.

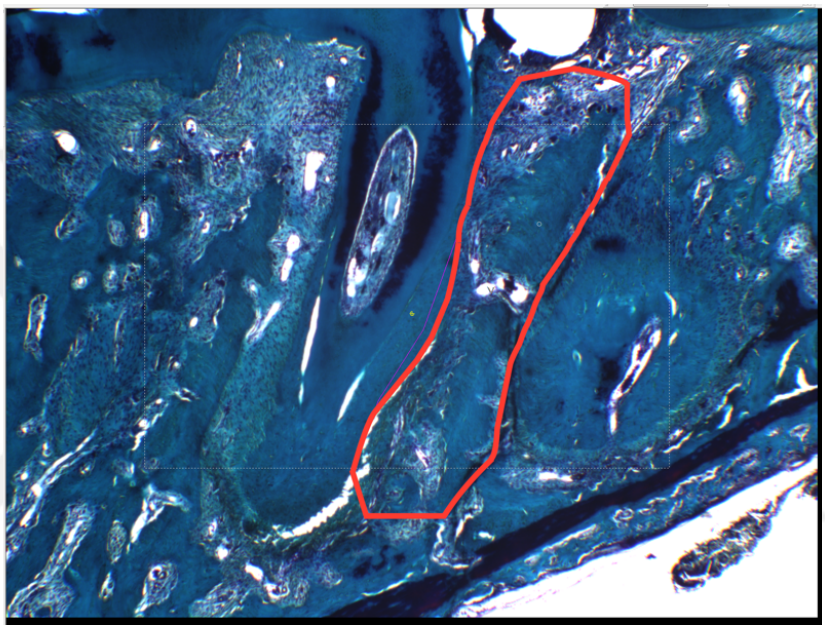

Fig 2.4. The bone area found in between the upper first and second molars, which is cervically limited by the connective tissue, apically by apical foramen and mesiodistally by the cements of 1st and 2nd molars, was investigated.

TRAP staining was used for detection of osteoclasts located in the bone. During the random sampling for the stereological method, the first section after the section in which roots of upper 1st and 2nd molars were most visible was used to evaluate TRAP+ activity. To count mesial osteoclasts, guiding gridline (20mm X 20 mm) was positioned at the mesial side of the mesial root of the upper 1st molar and TRAP+ osteoclasts were counted under 40X magnification. The distal osteoclasts were counted as in the previously described area between 1st and 2nd molars under 40X magnification.

3.2.2. Measurement of Orthodontic Tooth Movement Amount

Fig. 2.5. (a) Maxillary impression, (b) stone cast, (c) digital caliper for measuring the distance between upper 1st molar and 3rd molar

Orthodontic tooth movement was clinically assesed by measuring the distance between mesial cusp tip of upper first molar and mesial cusp tip of upper 3rd molar on initial and final stone casts with digital caliper (Fig. 2.5). The difference between the final and initial measurements were defined as orthodontic tooth movement amount. All measurements were made by the same investigator, who was blinded to the treatment groups and time points.

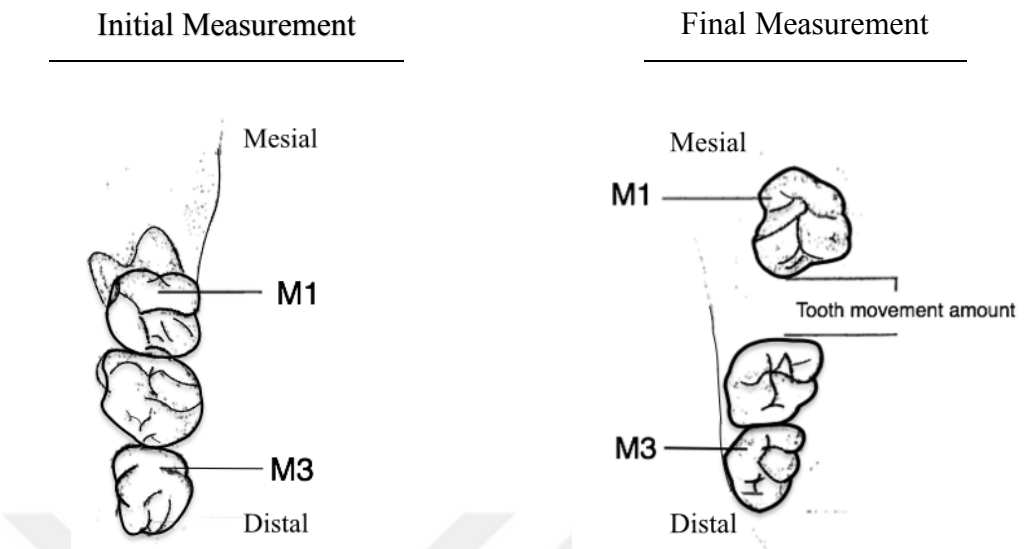

Fig. 2.6. The schematic diagram showing the clinical measurement of orthodontic tooth movement amount on stone casts

3.3 Statistical Evaluation

The statistical analysis of the histological and clinical results were made using *Statistical Package for The Social Sciences* version 18.0 software (SPSS Inc., Chicago, IL, USA). In each group, descriptive mean and standard deviation of each parameter were determined. One-way analysis of variance (ANOVA) test was used for the comparison of parameters between the groups. Post hoc Tukey HDS tests were performed to compare 2 groups of variables. A significance level of $P < 0.05$ was used for all statistical comparisons. The results were evaluated in confidence interval of 95%. The tests were carried out by blinding the operator to the groups and time points (by allocating new group codes by another investigator).

4. RESULTS

4.1. Reliability of Methodology

Table 4.1. Evaluation of reliability of methodology in orthodontic tooth movement measurements

Groups	Days	ICC[a]	p
Control	7	0,801	0.025*
	14	0,828	0.017*
	21	0,803	0.024*
1D	7	0,801	0.025*
	14	0,990	0,001**
	21	0,986	0.001**
3D	7	0,994	0,001**
	14	0,998	0.001**
	21	0,995	0.001**

*[a]ICC: Intraclass Correlation Coefficient, significance level * p<0.05,** p<0.001*

The results indicating reliability of methodology in orthodontic tooth movement measurements are listed in table 4.1. The intraclass correlation coefficient (ICC) for all groups is found to be close to 1.00, which indicates that the distances, that are measured at different times by the same investigator, have strong positive correlation between them. This has shown that the measurements made at different times are harmonious and accurate.

4.2. Observational Results

No problems in soft tissue healing was observed in decortication groups. Also, no signs of inflammation was observed in all groups.

In terms of weight loss, no significant difference was observed between control, single and three decortication groups on days 7 ($p=0.381$, $p>0.05$), 14 ($p=0.777$, $p>0.05$) and 21 ($p=0.897$, $p>0.05$).

Table 4.2. Weight loss in control and experiment group animals

Analysis of Variance Test	**CONTROL (Mean±SD) (g)**	**1D[a] (Mean±SD) (g)**	**3D[b] (Mean±SD) (g)**	***p****
7 DAYS	30.9 ± 8.3	35.6 ± 5.8	34.1 ± 61	0.381
14 DAYS	23.1 ± 7.5	25.8 ± 6.8	25.1 ± 8.7	0.777
21 DAYS	17.6 ± 8.1	18.6 ± 6.9	19.4 ± 7.4	0.897

**Significance level $p<0.05$, [a]1D: Single decortication group, [b]3D: three decortication group*

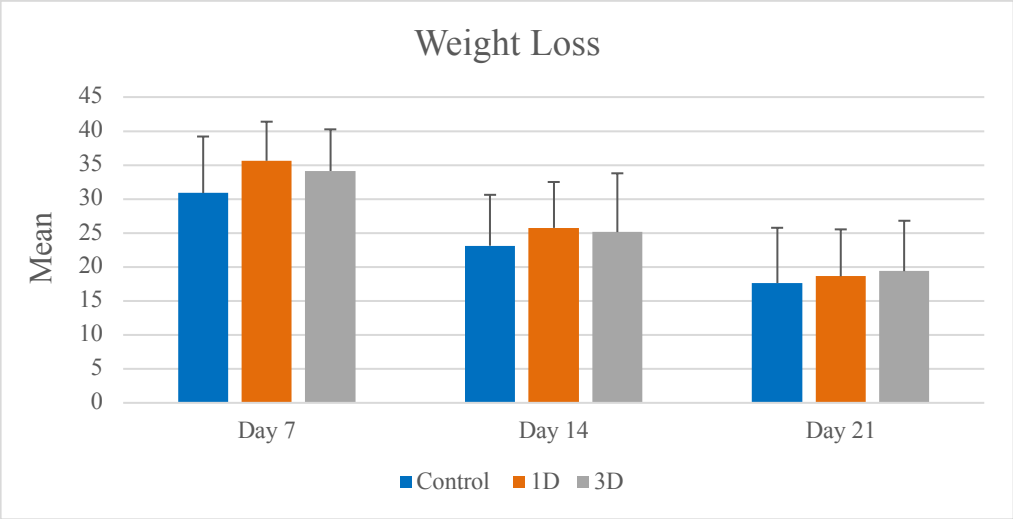

Fig. 2.7. Weight loss comparisons on days 7, 14 and 21 among the groups

4.3. Orthodontic Tooth Movement Amount

Orthodontic tooth movement amount in the control group showed statistically significant difference on days 7, 14 and 21 ($p=0.016$, $p<0.05$). In terms of orthodontic tooth movement amount, no statistical significant difference was observed between day 7 and day 14 ($p=0.407$, $p>0.05$). Also, no statistical significant difference was observed between day 14 and day 21 ($p=0.173$, $p>0.05$). However, the tooth movement amount on day 21 showed statistically significant increase compared to the tooth movement amount on day 7 ($p=0.012$, $p<0.05$).

Orthodontic tooth movement amount in the single decortication group presented statistically significant difference on days 7, 14 and 21 ($p=0.044$, $p<0.05$). No statistical significant difference was observed between day 7 and day 14 in terms of orthodontic tooth movement amount ($p=0.257$, $p>0.05$). In addition, no statistical significant difference was observed between day 14 and day 21 ($p=0.555$, $p>0.05$). However, the amount of tooth movement obtained in 21 days showed a statistically significant increase with respect to the amount of orthodontic tooth movement obtained in 7 days similar to the control group ($p=0.036$, $p<0.05$).

Table 4.3. Orthodontic tooth movement amount in control and experiment group animals

Analysis of Variance Test	**CONTROL**	**1D[a]**	**3D[b]**	***p****
	(Mean±SD) (mm)	**(Mean±SD) (mm)**	**(Mean±SD) (mm)**	
7 DAYS	0.545±0.113	0.53375±0.043	0.78±0.091	0.105
14 DAYS	0.7325±0.107	0.73125±0.051	0.915±0.051	0.157
21 DAYS	1±0.082	0.85875±0.133	1.18±0.153	0.221
***p**	**0,016***	**0,044***	**0,045***	

**Significance level p<0.05, [a]1D: Single decortication group, [b]3D: three decortication group*

Three decortication group manifested statistically significant difference on days 7, 14 and 21 in terms of orthodontic tooth movement (p=0.045, p<0.05). In terms of orthodontic tooth movement amount, no statistical significant difference was observed between day 7 and day 14 (p=0.653, p>0.05). Also, no statistical significant difference was observed between day 14 and day 21 (p=0.212, p>0.05). The amount of tooth movement observed in 21 days showed statistically significant increase compared to the amount of orthodontic tooth movement observed in 7 days similar to the previous two groups (p = 0.039, p <0.05).

However, when control, single and three decortication groups were compared, no statistically significant difference was found in terms of orthodontic tooth movement amount on days 7, 14 or 21 (p>0.05).

Table 4.4. Multiple comparison of OTM in experiment group animals

Post hoc Tukey Multiple Comparison Test	*p**
CONTROL 7/CONTROL 21	**0.012***
1D-7 / 1D-21	**0.036***
3D-7 / 3D-21	**0.039***
****Significance level p <0.05***	

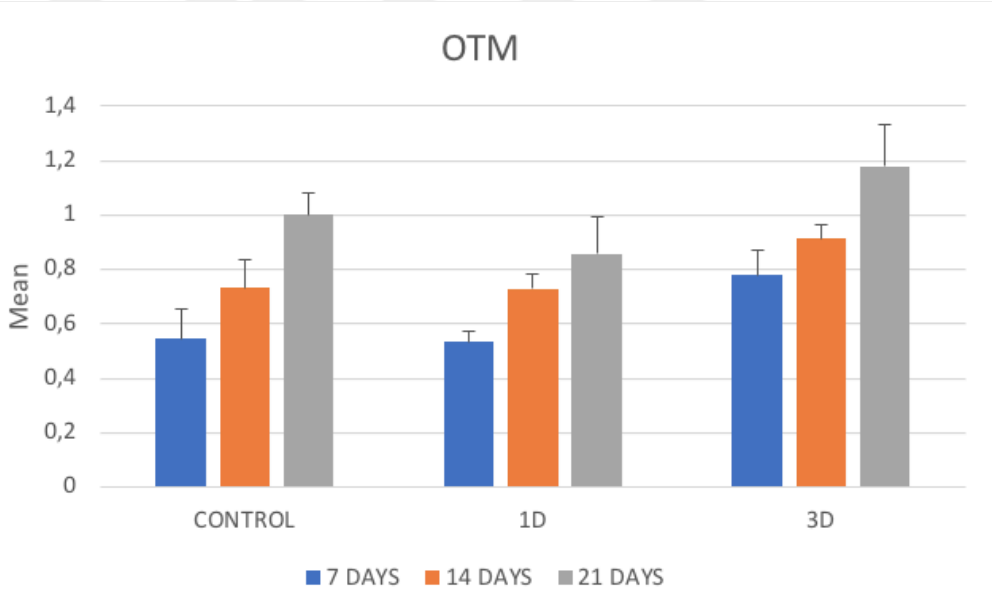

Fig. 2.8. Comparison of OTM amount among time points in control, single and three decortication groups

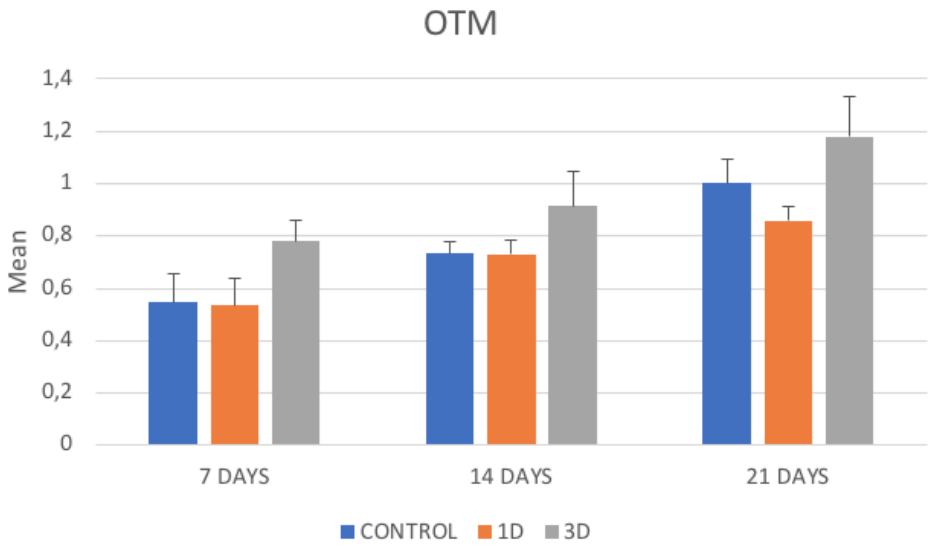

Fig. 2.9. Comparison of OTM amount among groups on days 7, 14 and 21

4.4. Histomorphometrical Findings

4.4.1. Bone Volume / Total Volume Ratio (BV/TV)

As a result of bone volume/ total volume ratio analysis of the alveolar bone between the upper 1st molar and 2nd molar, no statistically significant difference was found between control, single decortication and three decortication groups on days 7 and 14 ($p>0.05$).

However, on day 21, statistically significant difference was observed between these groups ($p=0.017$, $p<0.05$). In terms of bone volume / total volume ratio, no statistically significant difference was found between three decortication and control groups ($p=0.082$, $p>0.05$). Also, no statistically significant difference was observed between three decortication and single decortication groups ($p=0.457$, $p>0.05$) The decrease seen in the bone volume/total volume ratio in single decortication group was statistically significant compared to that of control group on day 21 ($p=0.015$, $p<0.05$). Intragroup changes observed in bone volume/ total volume ratio in single decortication and three decortication groups on days 7, 14 and 21 was not statistically significant ($p>0.05$). However, statistically significant difference was evident in the control group on day 7, day 14 and day 21 ($p= 0.018$, $p<0.05$). In the control group, statistically significant increase was seen in terms of bone volume/total volume ratio on day 21 compared to day 7 ($p=0.03$, $p<0.05$) and day 14 ($p=0.03$, $p<0.05$).

However, no statistically significant difference was observed on day 14 compared to day 7 according to bone volume/ total volume ratio (p=1.00, p>0.05).

Table 4.5. Bone volume/total volume ratio in control and experiment group animals

Analysis of Variance Test	**CONTROL (Mean±SD)**	**1D[a] (Mean±SD)**	**3D[b] (Mean±SD)**	***p****
7 DAYS	0.348±0.015	0.398±0.057	0.3675±0.023	0.638
14 DAYS	0.348±0.010	0.335±0.027	0.45±0.065	0.150
21 DAYS	0.525±0.068	0.315±.013	0.386±0.028	**0.017***
***p**	**0.018***	0.467	0.386	

**Significance level p<0.05, [a]1D: Single decortication group, [b]3D: three decortication group*

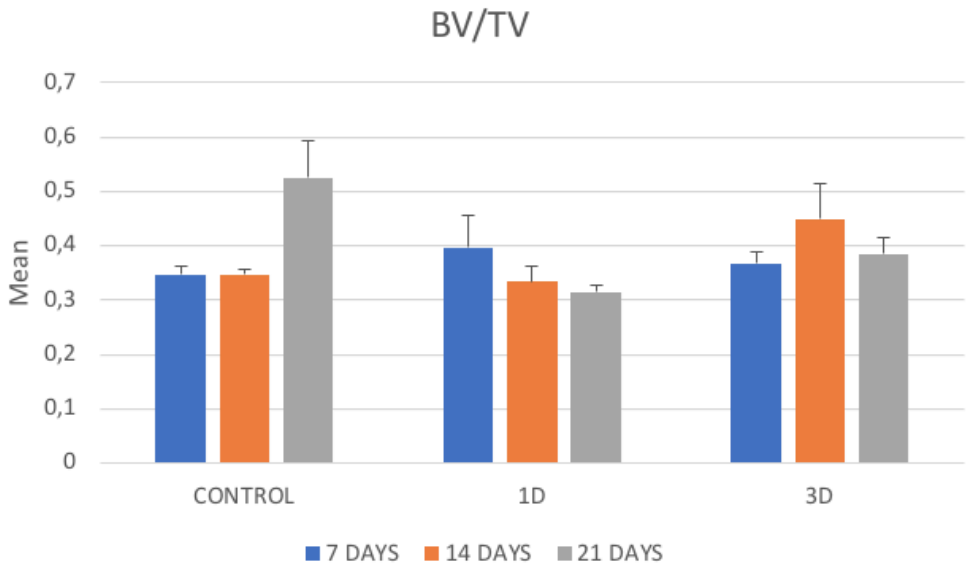

Fig. 2.10. Comparison of BV/TV ratio among time points control, single and three decortication groups

Table 4.6. Multiple comparison of BV/ TV ratio in control and experiment group animals

Groups	[1]*p*
CONTROL-7/ 1D-7	0.615
CONTROL-7 / 3D-7	0.922
1D-7 / 3D-7	0.834
CONTROL 14/ 1D-14	0.975
CONTROL 14 / 3D-14	0.235
1D-14 / 3D-14	0.173
CONTROL 21/ 1D-21	**0.015***
CONTROL 21 / 3D-21	0.082
1D-21 / 3D-21	0.457
CONTROL-7 / CONTROL-14	0.554
CONTROL-7 / CONTROL-21	**0.03***
CONTROL-14 / CONTROL-21	**0.03***
1D-7 / 1D-14	0.483
1D-7 / 1D-21	0.591
1D-14 / 1D-21	0.98
3D-7 / 3D-14	0.392
3D-7 / 3D-21	0.944
3D-14 / 3D-21	0.524

[1]Post hoc Tukey Multiple Comparison Test, [*]significance level p <0.05

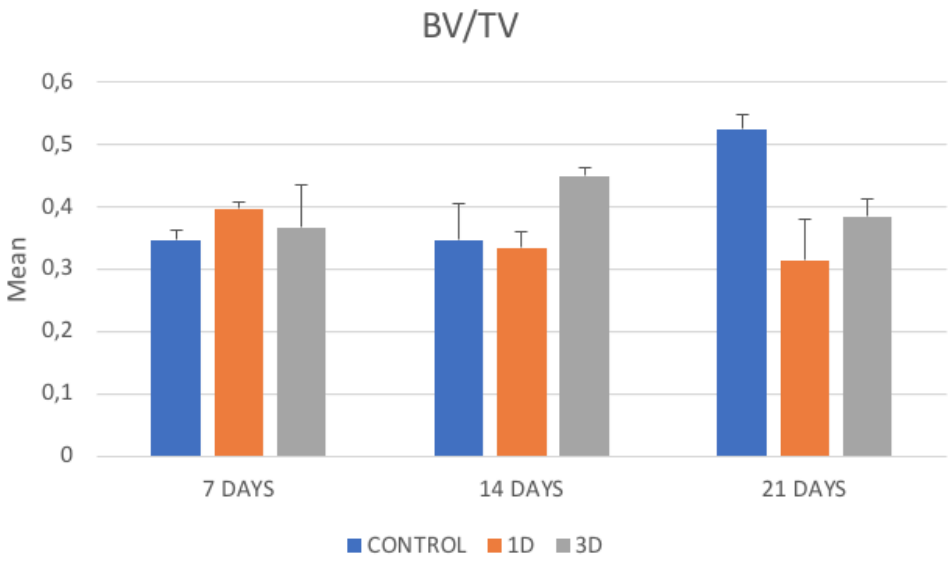

Fig. 2.11. Comparison of BV/TV ratio among the groups on days 7, 14 and 21

4.4.2. Bone Volume Fraction

Table 4.7. Results of bone volume fraction analysis in control and experiment group animals

Analysis of Variance Test	**CONTROL (Mean±SD)**	**1D[a] (Mean±SD)**	**3D[b] (Mean±SD)**	***p****
7 DAYS	0.255±0.009	0.34±0.029	0.2675±0.013	0.635
14 DAYS	0.26±0.004	0.25±0.014	0.3075±0,021	0.129
21 DAYS	0.34±0.028	0.233±0,010	0.282±0.014	**0.01***
p*	**0.012***	0.261	0.393	

**Significance level p<0.05, [a]1D: Single decortication group, [b]3D: three decortication group*

As a result of bone volume fraction analysis (BVF) of the alveolar bone between the upper 1st molar and 2nd molar, no statistically significant difference was found between control, single decortication and three decortication groups on days 7 and 14 (p>0.05).

However, on day 21, statistically significant difference was observed between these groups ($p=0.01$, $p<0.05$). In terms of (BVF), no statistically significant difference was found between three decortication and control groups ($p=0.118$, $p>0.05$). Also, no statistically significant difference was observed between three decortication and single decortication groups ($p=0.194$, $p>0.05$) The decrease seen in BVF in single decortication group was statistically significant compared to that of control group on day 21 ($p=0.008$, $p<0.05$).

Intragroup changes observed in BVF in single decortication and three decortication groups on days 7, 14 and 21 was not statistically significant ($p>0.05$). However, statistically significant difference was evident in the control group on day 7, day 14 and day 21 ($p= 0.018$, $p<0.05$). In the control group, statistically significant increase was seen in terms of BVF on day 21 compared to day 7 ($p=0.018$, $p<0.05$) and day 14 ($p=0.024$, $p<0.05$). However, no statistically significant difference was observed on day 14 compared to day 7 according to BVF ($p=0.978$, $p>0.05$).

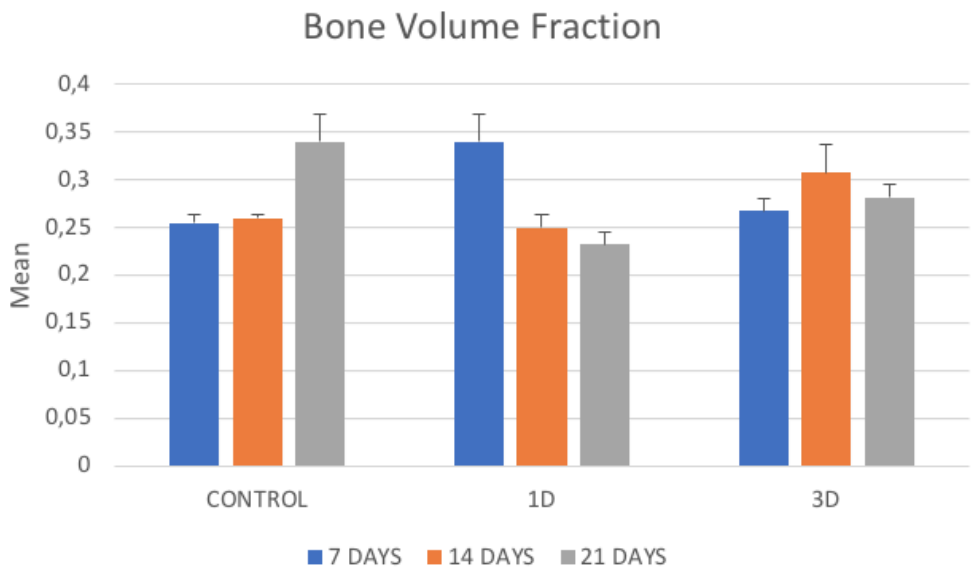

Fig. 2.12. Comparison of BVF values among time points in control, single and three decortication groups

Table 4.8. Multiple comparison of BVF results in control and experiment group animals

Groups	[1]*p*
CONTROL-7/ 1D-7	0.608
CONTROL-7 / 3D-7	0.878
1D-7 / 3D-7	0.878
CONTROL 14/ 1D-14	0.928
CONTROL 14 / 3D-14	0.236
1D-14 / 3D-14	0.138
CONTROL 21/ 1D-21	**0.008***
CONTROL 21 / 3D-21	0.118
1D-21 / 3D-21	0.194
CONTROL-7 / CONTROL-14	0.978
CONTROL-7 / CONTROL-21	**0.018***
CONTROL-14 / CONTROL-21	**0.024***
1D-7 / 1D-14	0.535
1D-7 / 1D-21	0.24
1D-14 / 1D-21	0.8
3D-7 / 3D-14	0.371
3D-7 / 3D-21	0.854
3D-14 / 3D-21	0.623

[1]Post hoc Tukey Multiple Comparison Test, [*]significance level p <0.05

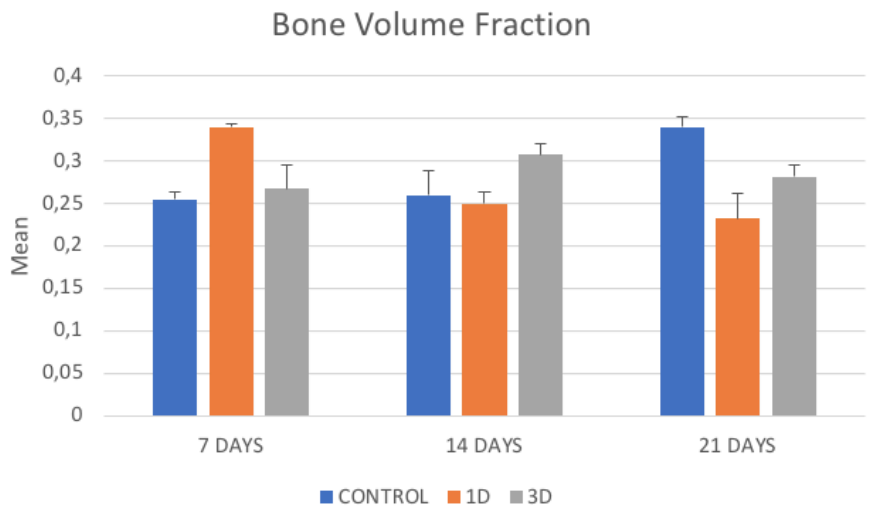

Fig. 2.13. BVF parameter comparison among the groups on days 7, 14 and 21

4.4.3. Total Volume Fraction

Table 4.9. Results of total volume fraction analysis in control and experiment group animals

Analysis of Variance Test	**CONTROL (Mean±SD)**	**1D[a] (Mean±SD)**	**3D[b] (Mean±SD)**	***p****
7 DAYS	0.745±0.009	0.72±0.027	0.7325±0.013	0.635
14 DAYS	0.7425±0.006	0.75±0.014	0.695±0.031	0.16
21 DAYS	0.66±0.029	0.7675±0.013	0.724±0.016	**0.013***
p*	**0.012***	0.261	0.453	

**Significance level p<0.05, [a]1D: Single decortication group, [b]3D: three decortication group[1]*

As a result of total volume fraction analysis (TVF) of the alveolar bone between the upper 1st molar and 2nd molar, no statistically significant difference was found

between control, single decortication and three decortication groups on days 7 and 14 ($p>0.05$). However, on day 21, statistically significant difference was observed between these groups ($p=0.013$, $p<0.05$).

In terms of TVF, no statistically significant difference was found between three decortication and control groups ($p=0.098$, $p>0.05$). Also, no statistically significant difference was observed between three decortication and single decortication groups ($p=0.297$, $p<0.05$) The increase seen in TVF in single decortication group was statistically significant compared to that of control group on day 21 ($p=0.01$, $p<0.05$).

Intragroup changes observed in TVF in single decortication and three decortication groups on days 7, 14 and 21 was not statistically significant ($p>0.05$). However, statistically significant difference was evident in the control group on day 7, day 14 and day 21 ($p= 0.012$, $p<0.05$). In the control group, statistically significant decrease was seen in terms of TVF on day 21 compared to day 7 ($p=0.019$, $p>0.05$) and day 14 ($p=0.022$, $p>0.05$). However, no statistically significant difference was observed on day 14 compared to day 7 according to TVF ($p=0.994$, $p>0.05$).

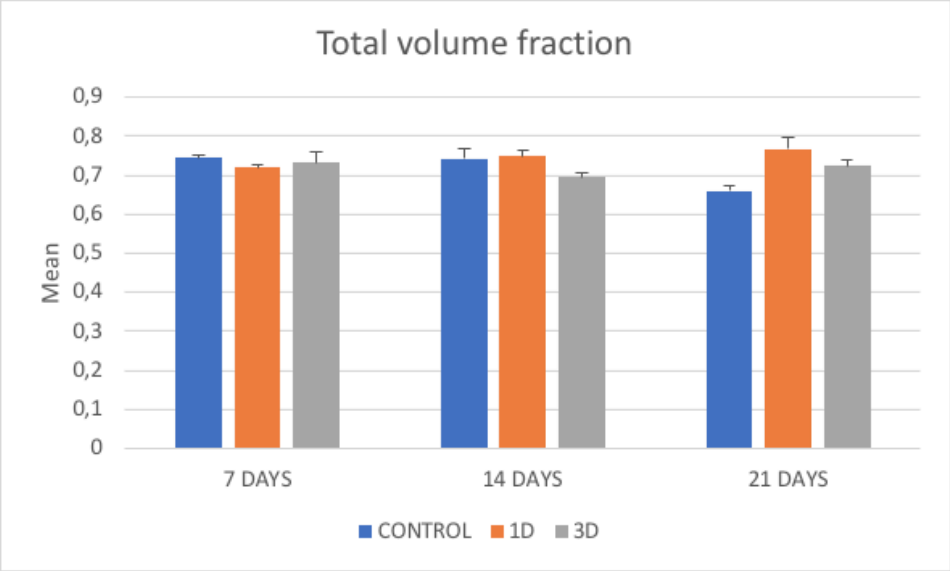

Fig. 2.14. Comparison of TVF results among the groups on days 7, 14 and 21

Table 4.10. Multiple comparison of TVF results in control and experiment group animals

Groups	[1]*p*
CONTROL-7/ 1D-7	0.608
CONTROL-7 / 3D-7	0.878
1D-7 / 3D-7	0.878
CONTROL 14/ 1D-14	0.962
CONTROL 14 / 3D-14	0.26
1D-14 / 3D-14	0.178
CONTROL 21/ 1D-21	**0.01***
CONTROL 21 / 3D-21	0.098
1D-21 / 3D-21	0.297
CONTROL-7 / CONTROL-14	0.994
CONTROL-7 / CONTROL-21	**0.019***
CONTROL-14 / CONTROL-21	**0.022***
1D-7 / 1D-14	0.535
1D-7 / 1D-21	0.24
1D-14 / 1D-21	0.8
3D-7 / 3D-14	0.458
3D-7 / 3D-21	0.953
3D-14 / 3D-21	0.587

[1]Post hoc Tukey Multiple Comparison Test, [*]significance level p <0.05

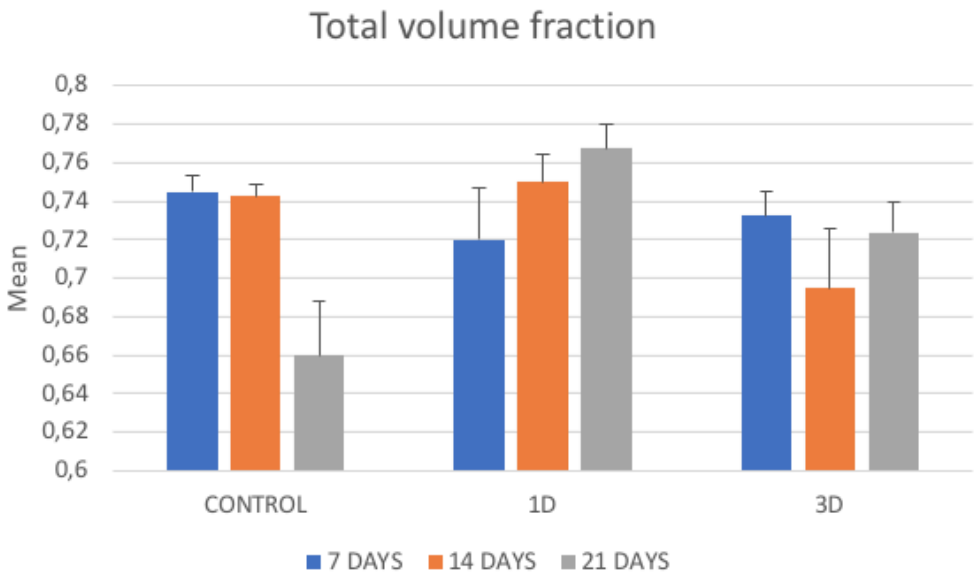

Fig. 2.15. Comparison of TVF results among time points in control, single and three decortication groups

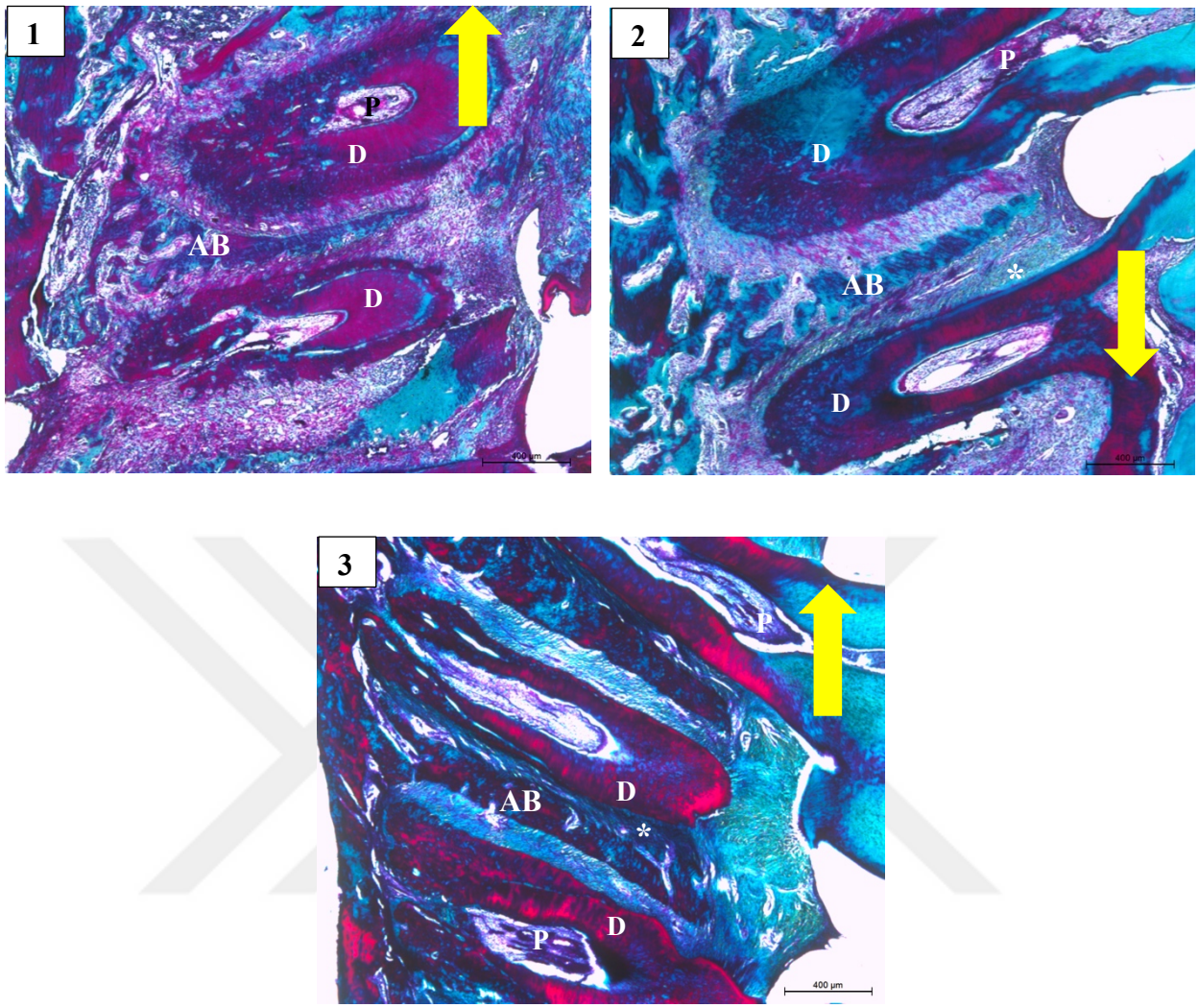

Fig. 2.16. Masson's trichrome stained histological sections showing alveolar bone of control **(1),** single **(2)** and three decortication **(3)** groups on day 7. Alveolar bone, dentin, pulp tissue and PDL space are indicated with AB, D, P and (∗) respectively. Yellow arrow indicates the direction of tooth movement. Note increased periodontal space and increase in bone porosity. Bar: 400µ, magnification: X5

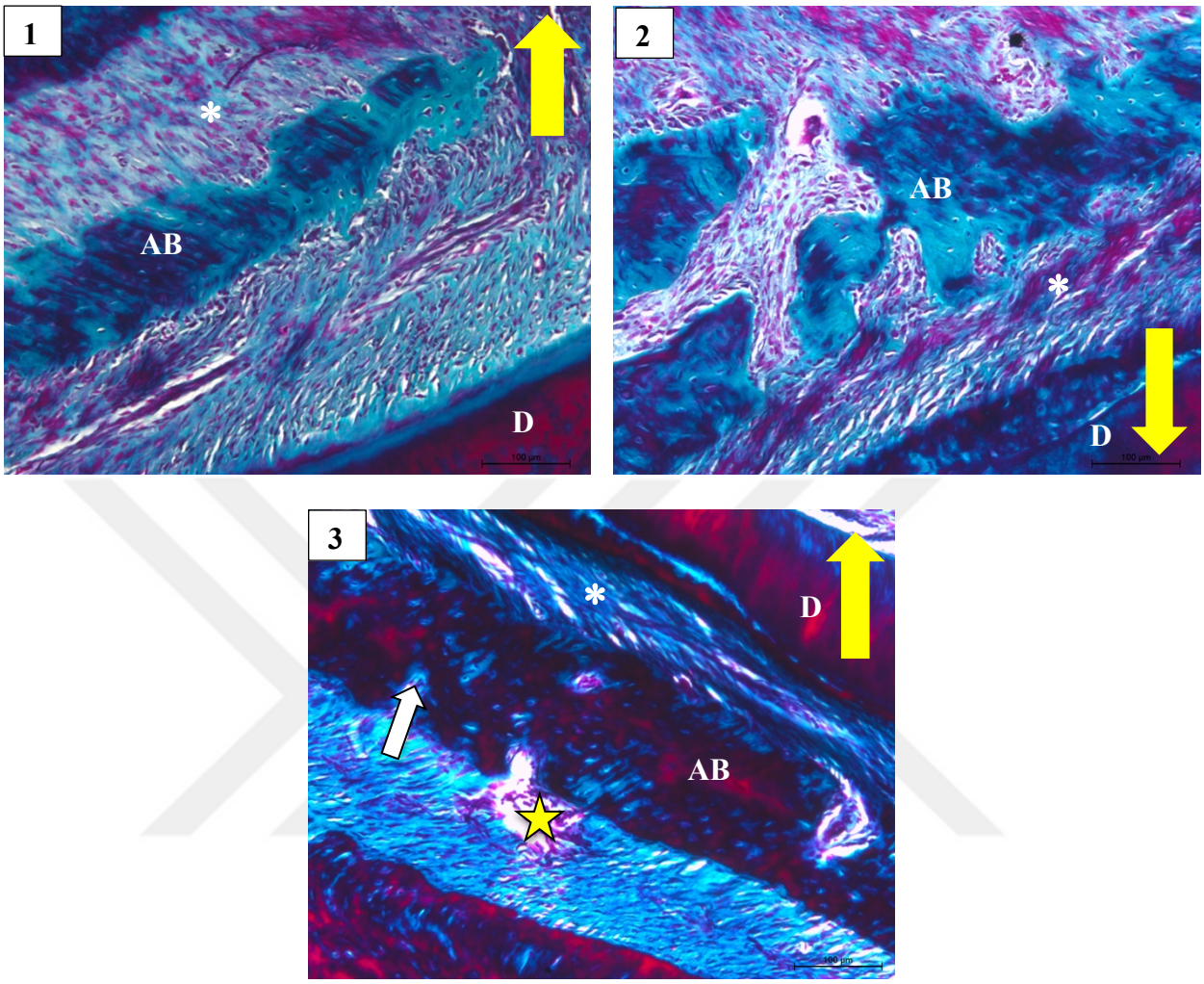

Fig. 2.17. Masson's trichrome stained histological sections showing alveolar bone of control **(1),** single **(2)** and three decortication **(3)** groups on day 7. Alveolar bone, dentin and PDL space are indicated with AB, D, and (*) respectively. Yellow arrow indicates the direction of tooth movement. Note new bone formation areas (arrow) and increase in bone vascularity (star). Bar: 100µ, magnification: X20

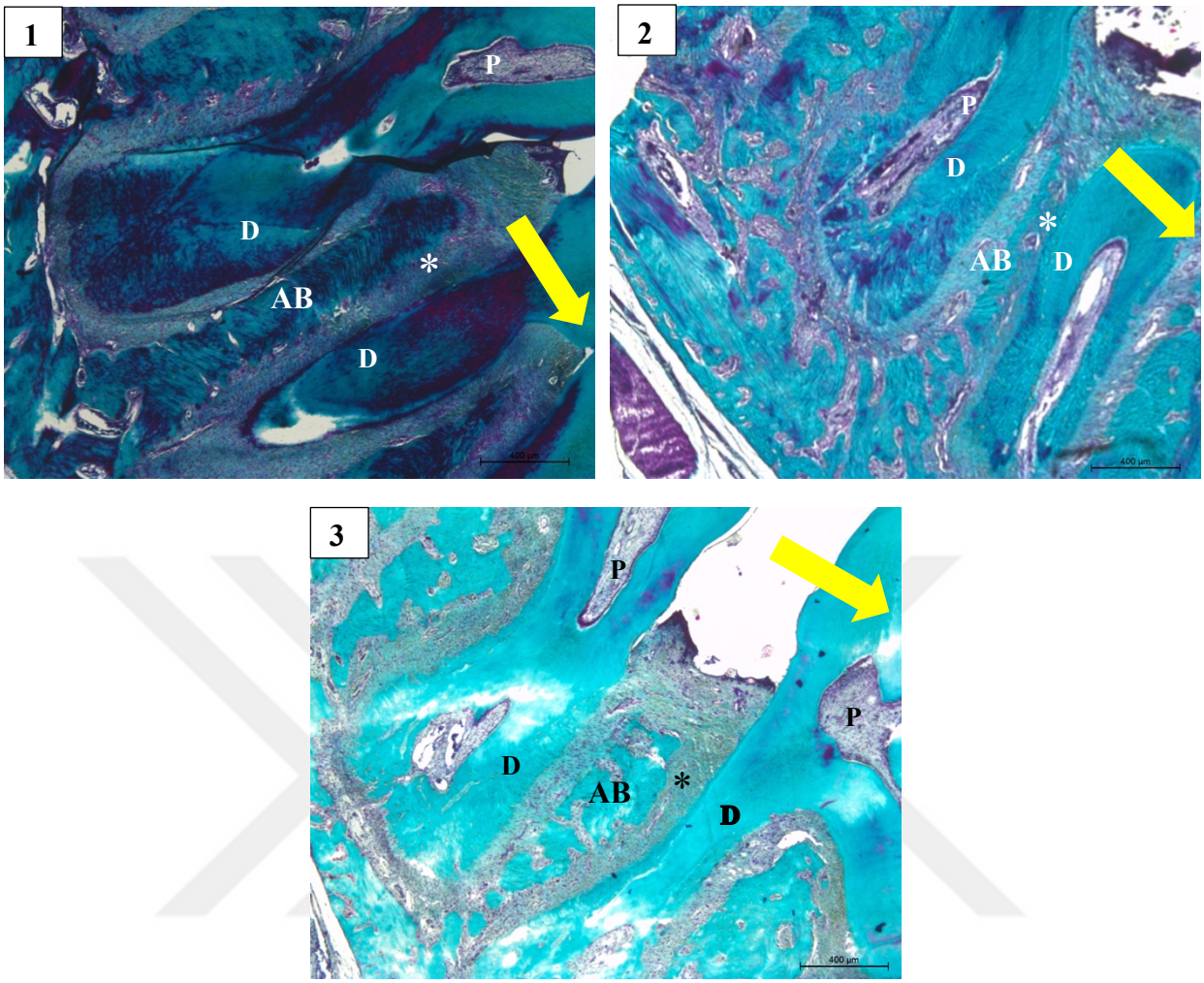

Fig. 2.18. Masson's trichrome stained histological sections showing alveolar bone of control **(1),** single **(2)** and three decortication **(3)** groups on day 14. Alveolar bone, dentin and pulp tissue are indicated with AB, D, P and (*) respectively. Yellow arrow indicates the direction of tooth movement. Note increased periodontal space and increase in bone porosity. Bar: 400µ, magnification: X5

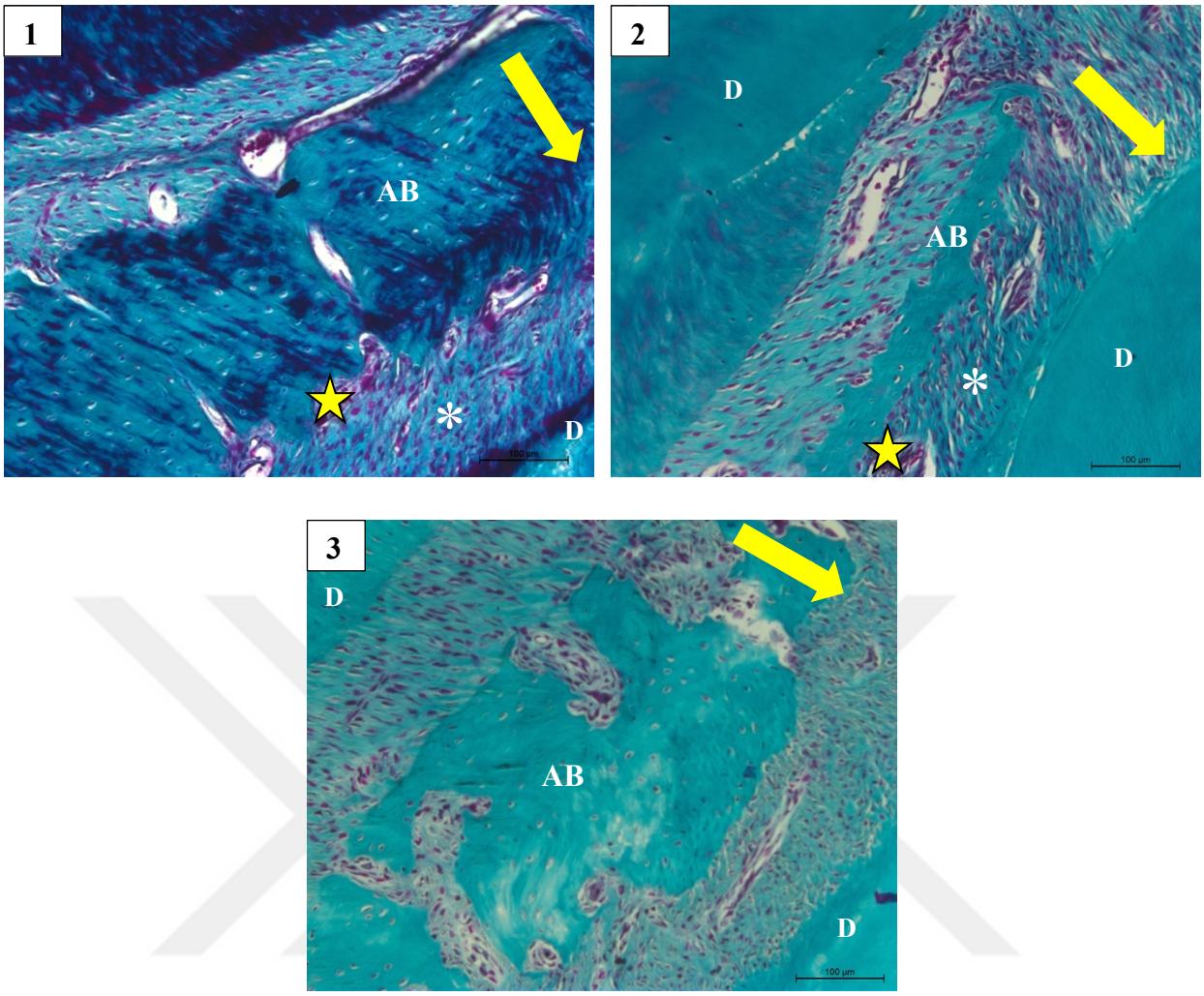

Fig. 2.19. Masson's trichrome stained histological sections showing alveolar bone of control **(1),** single **(2**) and three decortication **(3**) groups on day 14. Alveolar bone, dentin tissue and PDL space are indicated with AB, D and (∗) respectively. Yellow arrow indicates the direction of tooth movement. Note increased periodontal space and increase in bone vascularity (star). Bar: 100µ, magnification: X20

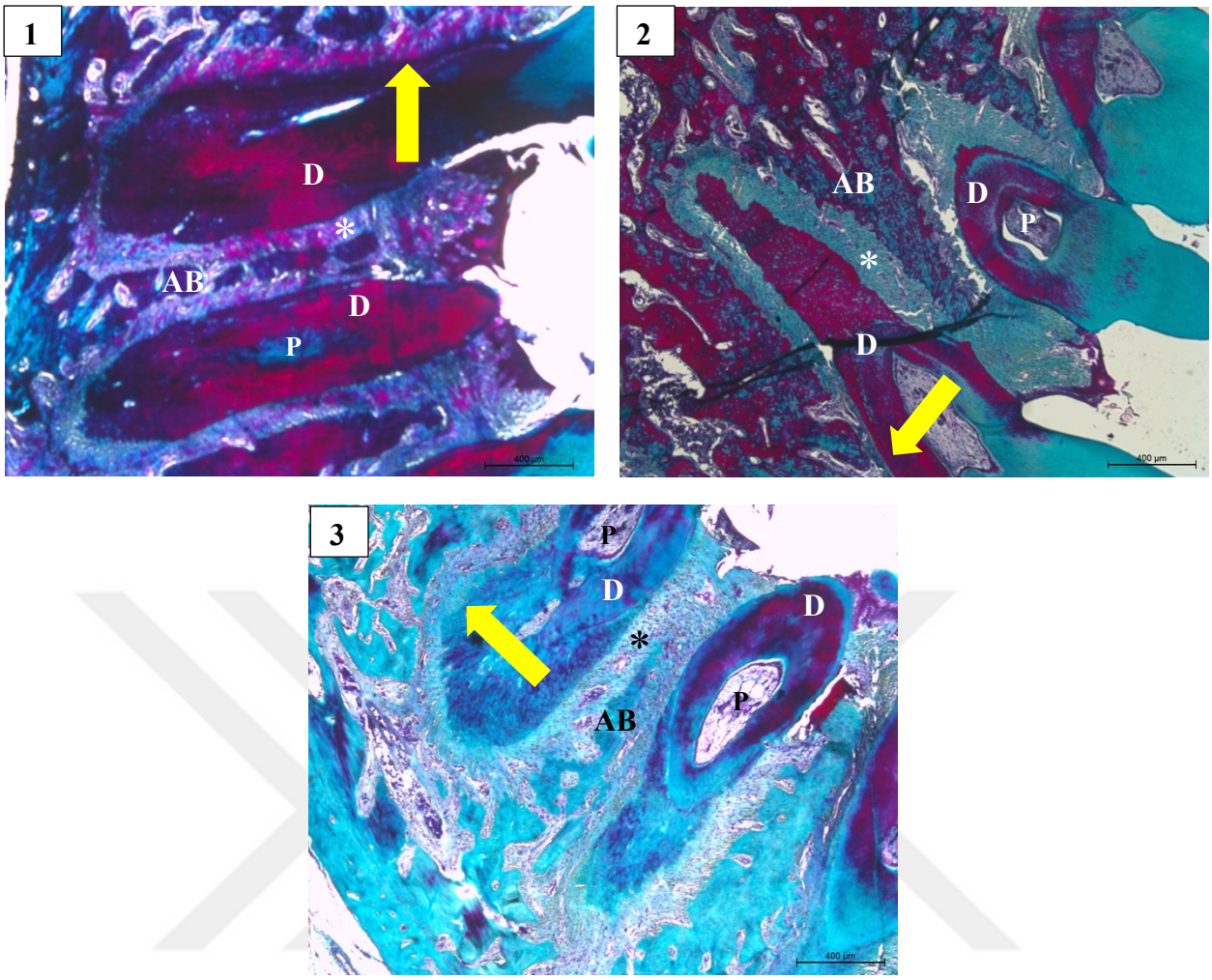

Fig. 2.20. Masson's trichrome stained histological sections showing alveolar bone of control **(1),** single **(2)** and three decortication **(3)** groups on day 21. Alveolar bone, dentin, pulp tissue and PDL space are indicated with AB, D, P and (∗) respectively. Note new bone formation areas and increase in bone vascularity. Bar: 400µ, magnification: X5

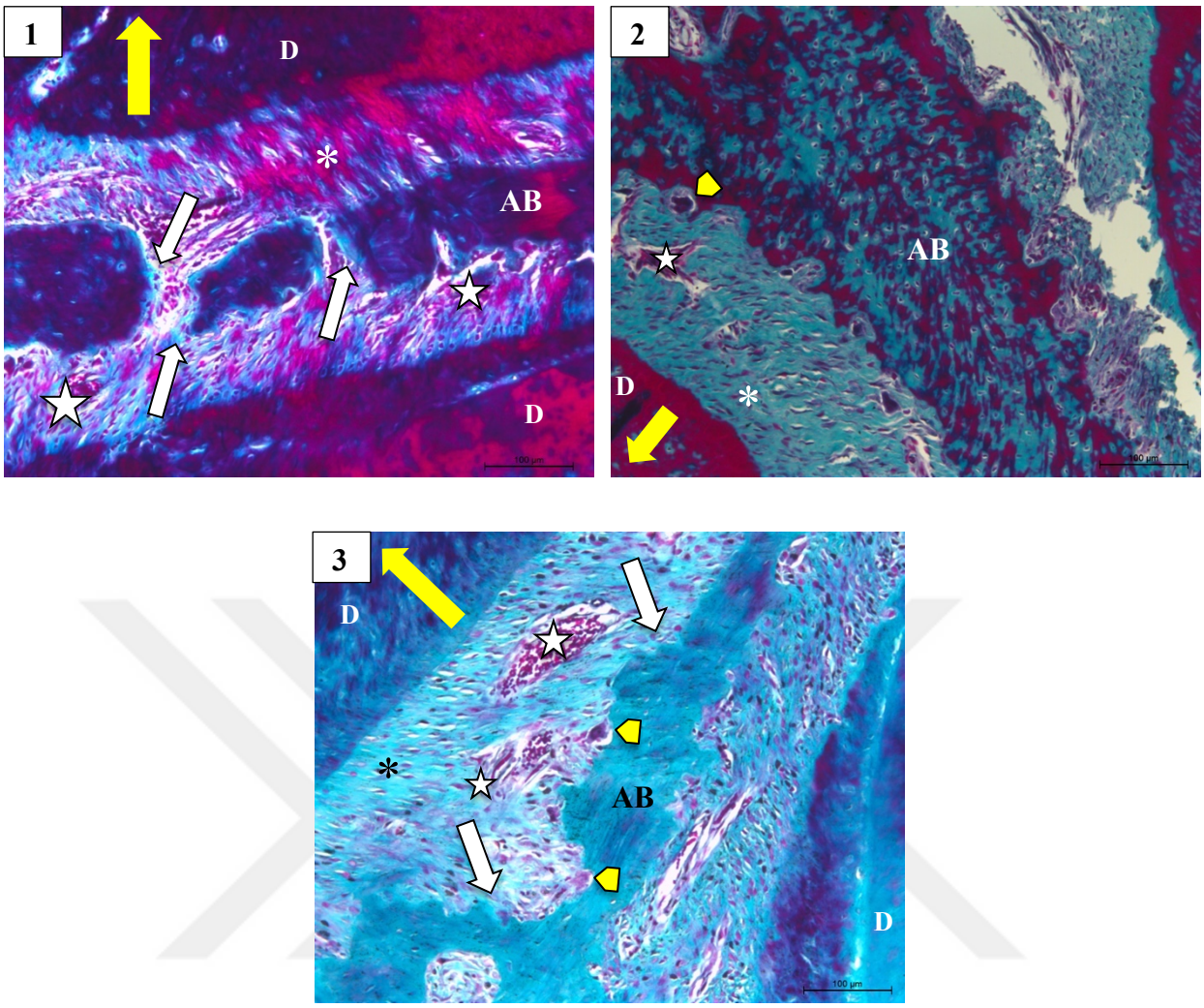

Fig. 2.21. Masson's trichrome stained histological sections showing alveolar bone of control **(1),** single **(2)** and three decortication **(3)** groups on day 21. Alveolar bone, dentin, and PDL space are indicated with AB, D, and (*) respectively. Note new bone formation areas (white arrow), increase in bone vascularity (star) and osteoclasts (yellow arrow head). Bar: 100µ, magnification: X20

4.4.4. Osteoclast Numbers on the Mesial Side of The Upper 1st Molar

Table 4.11. Mesial osteoclast numbers in control and experiment group animals

Analysis of Variance Test	**CONTROL**	**1D[a]**	**3D[b]**	***p****
	(Mean±SD)	**(Mean±SD)**	**(Mean±SD)**	
7 DAYS	17.25±1.493	16.25±0.853	21±0.913	**0.035***
14 DAYS	17.75±0.629	21.25±0.629	27.5±1.041	**0.00***
21 DAYS	19.75±1.109	21±1.291	26.25±1,031	**0.007***
p*	0.304	**0.008***	**0.003***	

**Significance level p<0.05, [a]1D: Single decortication group, [b]3D: three decortication group*

When osteoclast numbers, which are located mesially to upper 1st molar (compression side), were evaluated statistical significant difference was found between control, single decortication and three decortication groups on day 7 ($p=0.035$, $p<0.05$), 14 ($p=0.00$, $p<0.05$) and 21 ($p=0.007$, $p<0.05$). On day 7, while no statistical significant difference was observed between control and single decortication groups ($p=0.808$, $p>0.05$) as well as control and three decortication groups ($p=0.098$, $p>0.05$), osteoclast numbers in three-decortication group have shown statistically significant increase compared to single decortication group ($p=0.037$, $p<0.05$).

On day 14, statistically significant difference was seen between control, single decortication and three decortication groups ($p=0.00$, $p<0.05$). Osteoclast numbers in single decortication group have shown statistically significant increase compared to control group ($p=0.03$, $p<0.05$). Likewise, osteoclast numbers in three decortication group have shown statistically significant increase compared to control group ($p=0.00$, $p<0.05$). Moreover, the increase seen in three decortication group compared to single decoration group was statistically significant ($p=0.001$, $p<0.05$).

Osteoclast numbers of control, single decortication and three decortication groups showed statistically significant difference on day 21 ($p=0.007$, $p<0.05$). No statistically significant difference was observed between single decortication and control group ($p=0.73$, $p>0.05$). However, osteoclast numbers in three decortication group have manifested significant increase compared to control ($p=0.008$, $p<0.05$) and single decortication groups ($p=0.025$, $p<0.05$) on day 21.

When intragroup comparisons were made, no significant difference was found in the control group in terms of mesial osteoclast numbers at different time intervals ($p=0.304$, $p>0.05$).

Statistically significant difference was found in single and three decortication groups on days 7, 14 and 21 ($p= 0.0038$, $p=0.003$, $p<0.05$, respectively). In these group, osteoclast numbers on day 14 ($p=0.013$, $p=0.03$ $p<0.05$) and 21 ($p=0.017$, $p=0.012$ $p<0.05$) showed statistically significant increase compared to day 7. However, no statistically significant difference was observed between days 14 and 21 ($p=0.982$, $p=0.061$ $p>0.05$).

Table 4.12. Multiple comparison of mesial osteoclast numbers in control and experiment group animals

Groups	[1]*p*
CONTROL-7/ 1D-7	0.8
CONTROL-7 / 3D-7	0.098
1D-7 / 3D-7	**0.037***
CONTROL 14/ 1D-14	**0.03***
CONTROL 14 / 3D-14	**0.00***
1D-14 / 3D-14	**0.001***
CONTROL 21/ 1D-21	0.73
CONTROL 21 / 3D-21	**0.008***
1D-21 / 3D-21	**0.025***
CONTROL-7 / CONTROL-14	0.948
CONTROL-7 / CONTROL-21	0.311
CONTROL-14 / CONTROL-21	0.457
1D-7 / 1D-14	**0.013***
1D-7 / 1D-21	**0.017***
1D-14 / 1D-21	0.982
3D-7 / 3D-14	**0.003***
3D-7 / 3D-21	**0.012***
3D-14 / 3D-21	0.661

[1]Post hoc Tukey Multiple Comparison Test, [*]significance level p <0.05

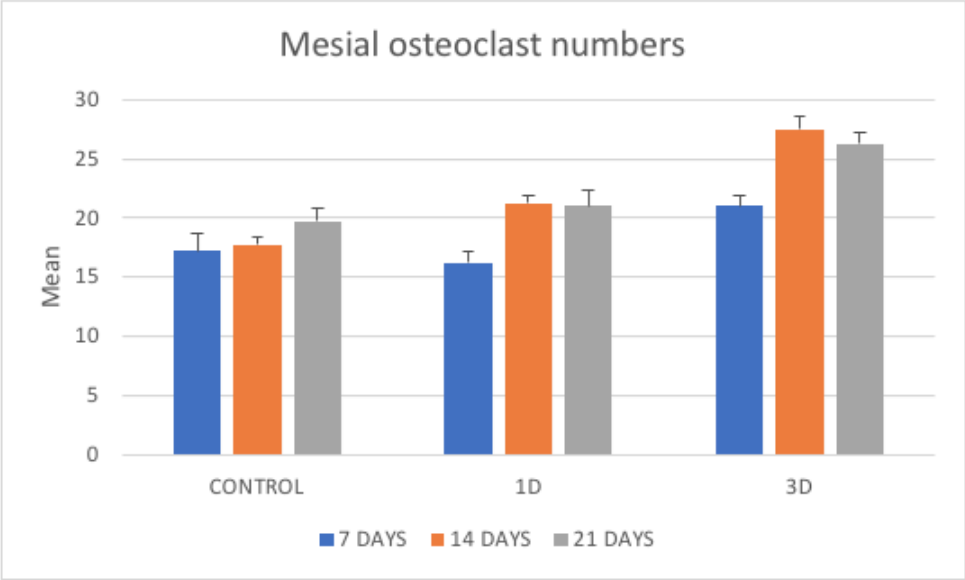

Fig. 2.22. Comparison of mesial osteoclast numbers among time points in control, single and three decortication groups

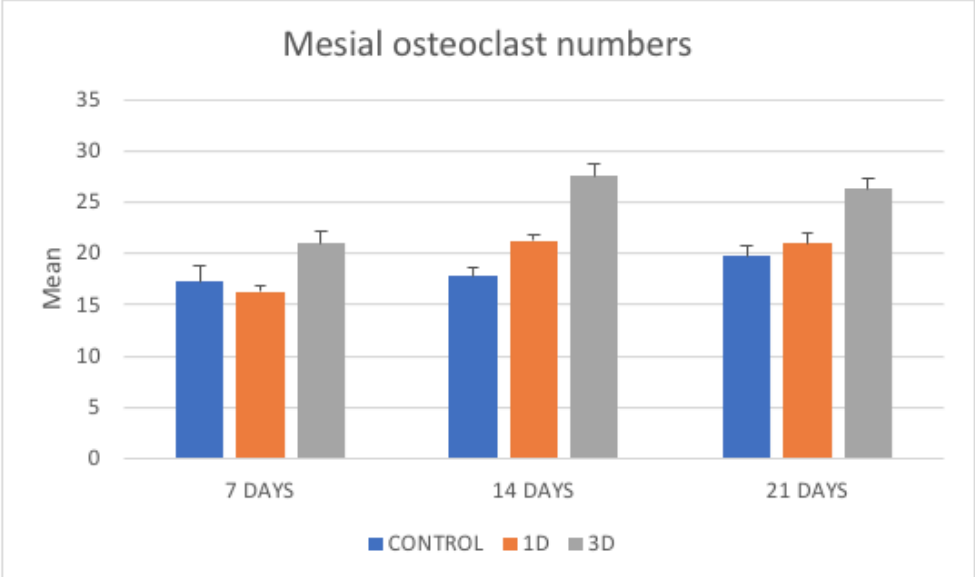

Fig. 2.23. Comparison of TVF results among the groups on days 7, 14 and 21

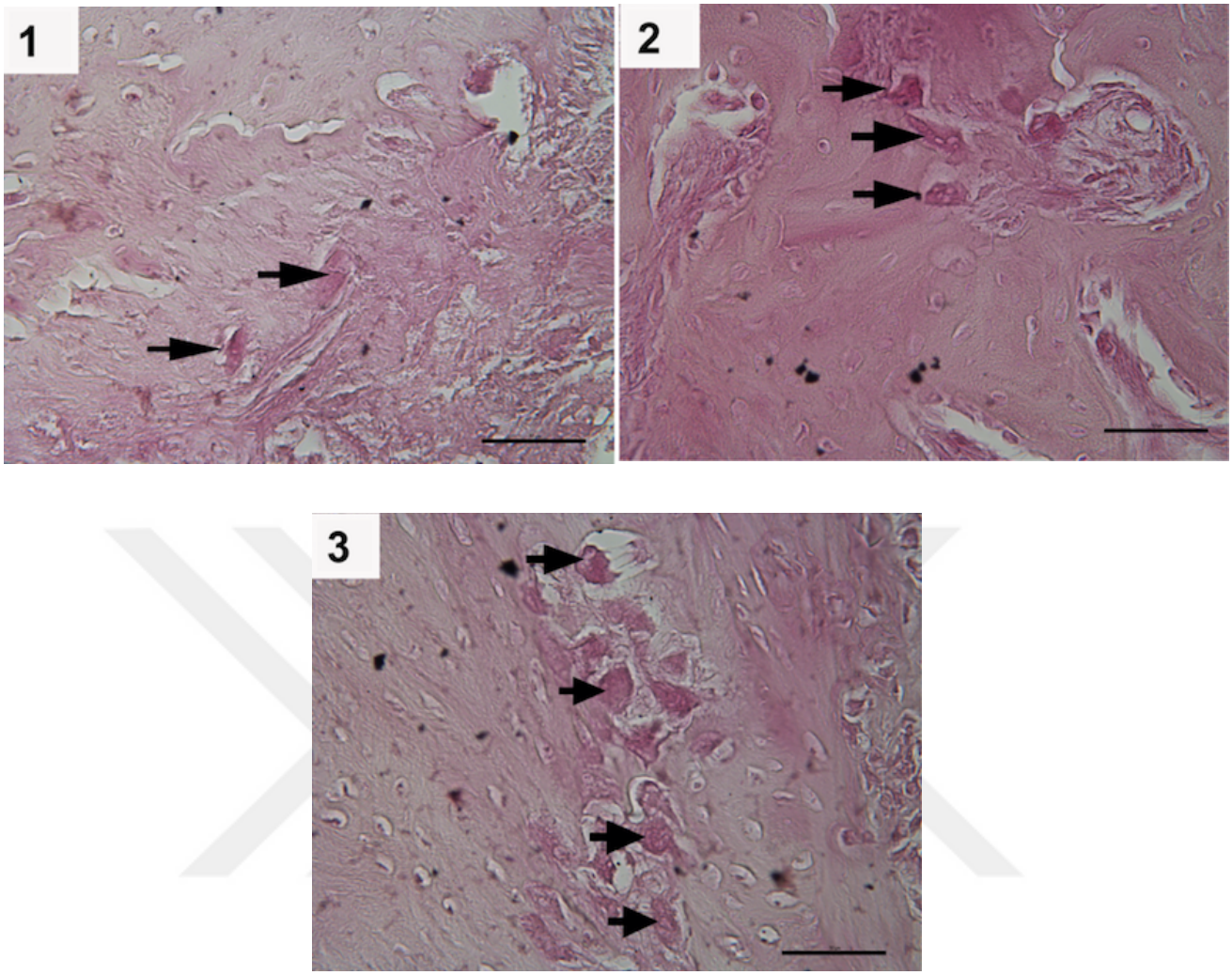

Fig. 2.24. Tartrate-resistant acid phosphatase stained histological sections showing mesial osteoclasts in control **(1),** single **(2**) and three decortication **(3**) groups on day 21. Arrows indicating TRAP + osteoclasts. Bar: 50μ, magnification: X40

4.4.5. Osteoclast Numbers on the Distal Side of the Upper 1st Molar

Table 4.13.: Distal osteoclast numbers in control and experiment group animals

Analysis of Variance Test	**CONTROL**	**1D[a]**	**3D[b]**	***p****
	(Mean±Std)	**(Mean±Std)**	**(Mean±Std)**	
7 DAYS	9.25±0.75	9.25±0.75	8.75±0.75	0.201
14 DAYS	11.5±0.646	14.25±0.479	16±0.479	**0.008***
21 DAYS	18.25±1.031	19.25±0.75	16.5±0.646	0.11
p*	**0.000***	**0.000***	**0.000***	

**Significance level p<0.05, [a]1D: Single decortication group, [b]3D: three decortication group*

When osteoclast numbers located at the area between distal root of the upper 1st molar and mesial root of the upper 2nd molar (tension side) were investigated, no statistically significant differences were found between control group, single decortication group and three decortication groups on days 7 (p=0.201, p>0.05) and 21 (p=0.11, p>0.05). On day 14, statistically significant difference was found between groups (p=0.008, p<0.05). Distal osteoclast numbers in three decortication group have shown statistically significant increase compared to control group (p=0.007, p<0.05). No statistical significant difference was detected between single decortication and control groups (p= 0.079, p>0.05) as well as three decortication and single decortication groups (p=0.298, p>0.05).

When intragroup comparisons were made distal osteoclast numbers in control, single and three decortication groups have shown statistically significant difference on days 7, 14 and 21 (p=0.00, p<0.05). In the control group, the osteoclast numbers on day 21 have demonstrated significant increase compared to day 7 (p=0.00, p<0.05).

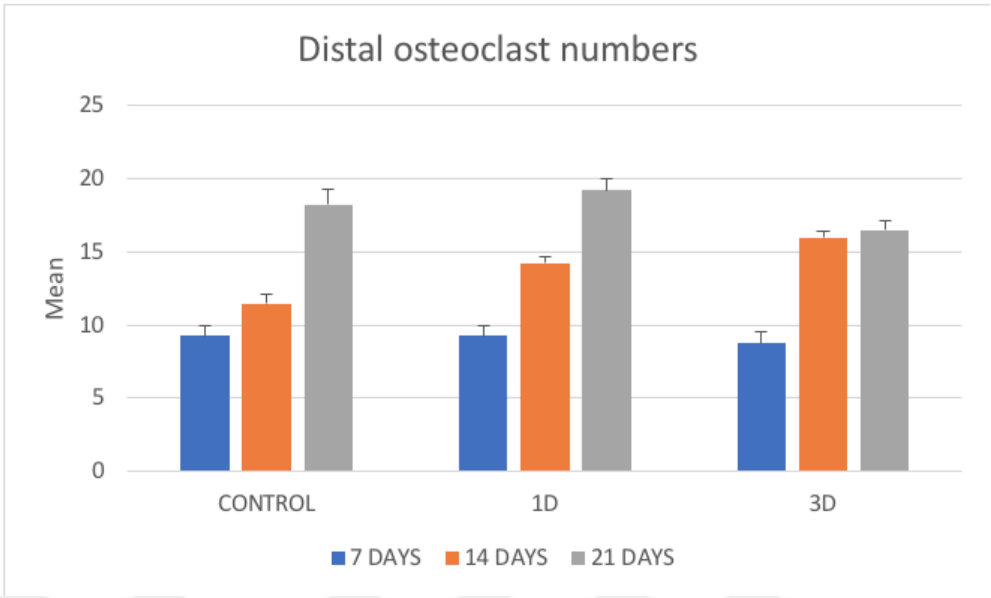

Fig. 2.25. Comparison of distal osteoclast numbers among time points in control, single and three decortication groups

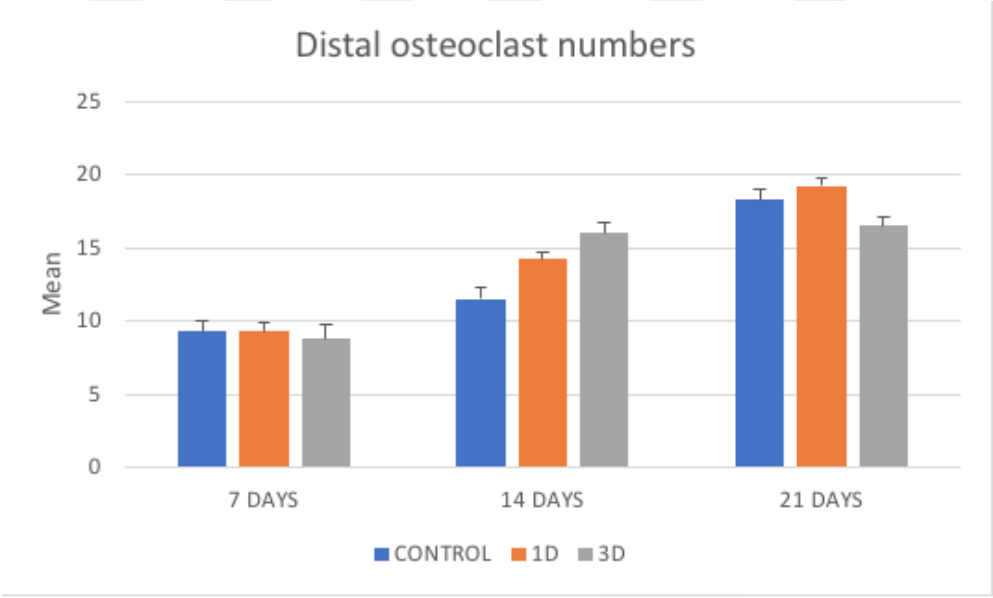

Fig. 2.26. Comparison of distal osteoclast numbers among the groups on days 7, 14 and 21

Also, the increase noted on day 21 was statistically significant compared to day 14 ($p=0.001$, $p<0.05$). However, in terms of distal osteoclast numbers, no statistically significant difference was seen between day 7 and day 14 in the control group ($p=0.186$, $p>0.05$).

Distal osteoclast numbers in single decortication have exhibited statistically significant difference between different time intervals ($p=0.00$, $p<0.05$). The distal osteoclast numbers have increased significantly on day 21 compared to day 7 ($p=0.00$, $p<0.005$) and day 14 ($p=0.001$, $p<0.05$). In addition, the distal osteoclast numbers on day 14 was found to be significantly higher than that of day 7 ($p=0.00$, $p<0.005$).

Statistically significant difference was observed in three decortication group on days 7, 14 and 21 ($p=0.00$, $p<0.05$). The distal osteoclast numbers on day 14 was found to be significantly higher than that of day 7 ($p=0.00$, $p<0.05$). The increase in osteoclast numbers on day 21 compared to day 7 was also statistically significant ($p=0.00$, $p<0.05$). However, when the osteoclast numbers in three decortication group on day 14 and day 21 were compared, no statistically significant difference was noted ($p=0.909$, $p>0.05$)

Table 4.14. Multiple comparison of distal osteoclast numbers in control and experiment group animals

Groups	[1]*p*
CONTROL-7/ 1D-7	0.198
CONTROL-7 / 3D-7	0.886
1D-7 / 3D-7	0.374
CONTROL 14/ 1D-14	0.079
CONTROL 14 / 3D-14	**0.007***
1D-14 / 3D-14	0.298
CONTROL 21/ 1D-21	0.679
CONTROL 21 / 3D-21	0.336
1D-21 / 3D-21	0.098
CONTROL-7 / CONTROL-14	0.186
CONTROL-7 / CONTROL-21	**0.00***
CONTROL-14 / CONTROL-21	**0.001***
1D-7 / 1D-14	**0.00***
1D-7 / 1D-21	**0.00***
1D-14 / 1D-21	**0.001***
3D-7 / 3D-14	**0.00***
3D-7 / 3D-21	**0.00***
3D-14 / 3D-21	0.909

[1]Post hoc Tukey Multiple Comparison Test, [*]Significance level p <0.05

5. DISCUSSION

The aim of this study was to evaluate clinical and histomorphometrical effects of the amount of alveolar decortication marks, which were made by slow handpiece, on orthodontic tooth movement in rats. In the current study, it has been desired to accelerate orthodontic tooth movement with a more conservative method, that is, without elevating the flap and giving less trauma to the alveolar bone.

Accelerating orthodontic treatment has been a need for both patients and for the orthodontists, to eliminate the side effects accompanying prolonged treatment. Since the duration of the orthodontic treatment is about 2-3 years (280), the attention of the researchers was driven towards the methods that will shorten this time span.

Orthodontic tooth movement is related with resorption and deposition of tooth socket. Resorption and deposition mechanisms seen in skeletal functional adaptation is also valid for orthodontic tooth movement (53). The rate of bone *turnover* determines the extent of orthodontic tooth movement (114). When the bone turnover rate is high tooth movement is accelerated, while tooth movement is slower as the turnover rate decreases.

Frost (126) implied that surgical wounding of the bone resulted in significant *remodelling* in soft and hard tissues adjacent to site of injury. All of these physiological events which were characterized with increased bone turnover and local osteopenia during healing was attributed as *regional acceleratory phenomenon (RAP)*

It was stated that orthodontic force alone could stimulate mild RAP reaction and RAP reached to maximum levels with corticotomy-assisted tooth movement (236).

Sebaun et al. (236) investigated the alveolar response particularly to corticotomy on rats. Following buccal and lingual full-thickness flap elevation, 10 decortication marks on the buccal and lingual aspects of alveolar bone were performed by means of 0.2 mm round bur and slow handpiece. The study design excluded orthodontic tooth movement. At the end of 3 weeks, the catabolic activity and anabolic activity was found to be increased in the surgery group.

Teixeira et al. (282) suggested that the method for corticotomy should be further simplified to reduce its harmful side effects. For this purpose, the authors preferred making 3 decortication marks following soft tissue flap elevation on their experimental study. It was noted that osteoclast numbers and orthodontic tooth movement amount was increased with generalized osteopenia being observed in the experiment group.

In the literature, many studies reported that orthodontic tooth movement was accelerated by corticotomy (240-42, 244-61, 308-09). Although most of the surgical methods for accelerating tooth movement involve elevation ofthe flap, researchers have seeked flapless surgical approaches to accelerate orthodontic tooth movement.

Kim et al. (263) introduced ‘‘Corticision’’ as minimal surgical intervention to accelerate tooth movement. The technique consisted of inserting the surgical blade through the gingiva and gradually penetrating it to cancellous bone and mobilizing the interproximal cortex. In their experimental study on cats, the authors concluded that bone resorption was increased with less hyalinised areas in the experimental group and hyalinised tissue was removed more rapidly in this group.

Dibart et al. (264) introduced the piezocision technique, in which corticision cuts were made going through the buccal vertical cuts by means of ultrasonic wave. The method also enabled soft and hard tissue grafting by tunnel approach. The authors claimed that treatment time was shortened with piezocision and the method had high patient acceptance.

Seifi et al. (277) performed flapless corticotomy with the help of Er, Cr: YSGG laser on New Zealand rabbits. Bone cutting was performed underwater-spray cooling in a noncontact manner with the area. They concluded that the amount of orthodontic tooth movement on the experiment side was significantly higher than the control side after 21 days. They suggested that laser assisted flapless corticotomy is useful procedure for reducing treatment time and damage to periodontium.

In 2017, Kurohama et al. (297) performed corticotomy-assisted tooth movement under two different force magnitudes (10 g and 25 g) in 56 rats and investigated the effect of bone volume removed during corticotomy on orthodontic tooth movement in 56 rats. After elevating full-thickness flaps, the volumes of the bone cut by corticotomy were 0.1, 1.0, and 1.7mm^3 in the 25g-force groups, and 1.0 and 1.7mm^3 in the 10g-force groups. At the end of 21 days, they concluded that the volume of the alveolar bone cut during corticotomy had no effect on tooth movement or root resorption; however, alveolar bone loss might be increased following tooth movement, especially resulting in reduced height of the alveolar bone.

Even though the effect of alveolar decortication amount was previously investigated in the literature, since it is hard to precisely measure the volume of bone removed in terms of mm^3, in our study we aimed to evaluate the effect of alveolar

decortication amount, in terms of number of decortication marks, on orthodontic tooth movement in rats

The decortication marks in the current research were made transmucosally at the buccal and palatal cortical bone located 5 mm mesial to the upper 1st molar. The perforations were performed by using 0.8 mm round bur and slow handpiece. The bur was advanced through the cortical thickness until the trabecular bone was reached. In the single decortication group, perforation was performed on the crest of the alveolar bone while on the three-decortication group, in addition to alveolar crest, buccal and palatal cortical bones received perforations.

Being first in the literature to perform selective alveolar decortication on animals, Sebaun et al. (236) performed 10 decortication marks on the buccal and lingual aspects of alveolar bone by means of 0.2 mm round bur and slow handpiece. Researchers preferred elevating buccal and lingual full-thickness flaps. In their study in 2010, Teixeira et al. (282) elevated mucoperiosteal flap around the upper 1st molars of 48 rats and performed 3 shallow perforations (0.25 mm depth and width) on the palatal cortex which were located 5 mm away from the upper 1st molars. Following elevation of buccal and palatal full-thickness flaps, Baloul et al. (251) made 5 perforations on each buccal and palatal cortical surface by means of slow handpiece and 0.25 mm bur. Meanwhile, similar to the present study, Tsai et al. (281) made transmucosal micro-osteoperforations on the cortical bone via slow handpiece. Researchers, working on 45 rats, made perforations on the palatal cortical bone 5 mm mesial to the upper 1st molar by using 0.25 mm round bur. One of the recent studies in this field was made by Cheung et al. (304). In their *split-mouth* study, the authors applied orthodontic force bilaterally by close coil springs. On the experiment side, 5 decortication marks, 1-3 mm apart from each other, were performed on the mesial and palatal aspects of upper 1st molar. The perforations were made by advancing mini-implant (1.2 mm diameter) 1 mm into the alveolar bone. Mini-implant driver with 30 rpm constant torque was used to replace the implant. All the insults were made in transmucosal approach.

In the current study, rats were used as the experimental animal model. Male rats were preferred in order to eliminate hormonal changes associated with estrous cycle.

The reason why rats are preferred in scientific studies can be listed as follows: they can be produced quickly, are easy to handle and conduct experiments on, can easily adapt to new environments, can be formed groups with similar characteristics in a short time and their costs are low (8). In addition, rats also enable investigation of human

biological phenomena which are not possible to study clinically due to ethical reasons (236).

The genetic strain of the rats used in this study is outbred. In other words, even though the rats look uniform, each rat is genetically different, which creates individual variation for each of the animal and render to respond them differently to the treatment. This might lead to considerable amount of variation in the results between the animals in the same group. This can be considered as a limitation of this study. From another perspective, this diversity in the genotype of the animals reflect the heterogeneity in the population of the orthodontic patients, which renders animal experiments valuable in clinical aspect.

Even though the mechanisms look alike in humans and rats following orthodontic force application, partial differences are present. Alveolar bone of the rats lack bone marrow spaces and osteons. Calcium metabolism is controlled by intestinal absorption rather than the bone tissue itself. When all of these come together, it can be concluded that the bone density of the rats is higher than that of humans. Remodelling activity is also faster than that of humans. The formation of cell-free hyalinised areas is observed as early as 6 hours after application of orthodontic force (8). Besides, it was stated that periodontal space was diminished after application of long-term heavy forces. It was also claimed that lack of osteoid tissue can give rise to delayed bone deposition (7).

In the present study, 16-week old Sprague Dawley rats were used. In a study comparing the effects of the ages of rats on the turnover rate of alveolar bone, under normal physiological conditions, the rate of turnover decreased rapidly between 6-week old and 30-40-week old rats and decreased even more rapidly in 50-100-week old of rats (291). In the present study, 16-week old adult rats were preferred both in terms of ease of working in the mouth and having greater physical endurance compared to young rats.

In the current study, parallel to many studies, the control and experiment groups were selected from separate experimental animals (236, 243, 245, 251, 279, 281, 296, 310-11). Following the placement of the appliance, the disorder in eating habits of the animals causes a temporary weight loss at first. The resulting physiological stress may, however, affect the bone response (305). In addition, the change in chewing activity which accompanies the appliance placement may affect the mechanical load distribution and can affect the control side as well as the experimental side (305,306). When all of the mentioned factors are taken into consideration, this study was not designed as *split-mouth.*

Due to rapid absorption and ease in application, the animals were anesthetized intraperitoneally at the beginning of the experiment prior to orthodontic force application. Accordingly, following injection of 10% Ketasol® (60 mg/kg/im, including 100 mg/ml ketamine hydrochloride, Richter Pharma AG, Wels, Austria), 2% Rompun® (5 mg/kg/im, including 23,32 mg/ml xylazine, Bayer HealthCare LLC., Kansas, USA) was injected to relax the muscles and avoid spontaneous movements during the intervention.

In our study, it was preferred to use Sentalloy closed springs with a force of 50 gr, which is considered to obtain sufficient tooth movement rather than heavy force, which produces lag phase.

Sentalloy close coil springs are fabricated to deliver constant and continuous force at a certain activation range. Ren et al. (292) concluded that Sentalloy springs give constant, continuous and repeatable force in the activation range of 3-15 mm. Similarly, Baloul et al. (251) stated that these springs apply constant and continuous force in the activation range of 10 mm. Moreover, Erdem (279) also preferred to use Sentalloy close coil springs to apply continuous force in her thesis study investigating the effects of Er:YAG laser decortication on orthodontic tooth movement in rats.

In the experimental orthodontic studies, elastic separators are placed between 1st and 2nd molars for orthodontic force application (295). Even if the method seems advantageous at first glance, owing to its ease in application, this method is not preferred due to the inability of the elastics to apply continuous and controlled force (292). It has been suggested that constant and continuous force application should be the priority of the experimental researches since intermittent and uncontrolled force renders the interpretation of the relationship between force and tooth movement difficult (295).

In the present study, 50 gr-force was applied to mesialize upper 1st molars. In order to confirm the accuracy of the applied force, the force applied by the closed coil spring was measured each time by means of a dynamometer.

When the rat orthodontic studies are examined, it is seen that the magnitude of the force applied to molar teeth varies. While Lee et al. (245) prefer using 100 gr-force, majority of the researchers considered using lighter forces. Goldie et al. (179) applied mesial force of 60 gr-force. Gonzales et al. (303); revealed that 10, 25 and 50 gr-force applications ended up with greater orthodontic tooth movement. While some authors used 50 gr-force for molar mesialization (281,301), in some studies molar mesialization was performed by lighter force magnitudes as10 gr-force (190, 210-13), 25 gr-force (92, 231, 251) or 30 gr-force (166, 176, 257).

In our study, rat molar teeth were used to measure orthodontic tooth movement amount. Rat incisors are not preferred to evaluate orthodontic tooth movement due to their continuous eruption and morphological difference (292). Since the buccal bone volume is more limited compared to mesial bone, and buccal bone has more compact structure, evaluation of molar mesialization as orthodontic tooth movement is found to be more accurate in rats.

In the current study, maxillary impressions were taken from the rats by polysiloxane impression material (Express XT Quick, 3M ESPE AG, Seefeld, Germany) at the beginning (initial) and after sacrification (final) of the animals. Afterwards, plaster models were obtained by means of plaster material (Unibase-300, Dentona AG, Dortmund, Germany), which was introduced to have coefficient of expansion factor less than 0.08% by the manufacturer. The orthodontic tooth movement was evaluated on dental stone casts. The measurement points were determined as mesial cusp tip of first molar tooth and mesial cusp tip of third molar tooth. Since the distance to be measured on the casts is small, the precision gains importance in measurements. Precise measurements were made on these models by digital calliper with an accuracy of 0.01 mm. Measurements were repeated twice by the same researcher. The measurements were carried out by blinding the operator to the groups and time points via allocating new group names by another investigator. When the measurements made at different times were found to have strong correlation with each other (ICC, $r>0.079$). The orthodontic tooth amount was assessed as the difference between the initial and final measurements.

When the studies investigating the orthodontic tooth movement in rats are evaluated, it is seen that the studies measuring the distance between the marked points on the upper first molar and second molar are abundant (166, 210, 213, 230). However, Verna et al. (114) and Dunn et al. (190) preferred to measure the distance between the mesial side of the first molar and distal side of the third molar. Likewise, Erdem (279) evaluated upper 1st mesialization amount by measuring the distance between the mesial cusp tip of upper first molar and distal cusp tip of the third molar. In the current study, following the first molar tooth movement, it was considered that the second molar tooth could move in the same direction due to the effect of periodontal fibres, and it was deemed appropriate to measure a wider distance in order to obtain more accurate results with the digital calliper.

Considering similar reasons and the potential failure in determining the distal cusp of the third molar due to deformation in the impression, it was found more suitable to

measure the distance between the mesial cusp tip of the first molar and the mesial cusp tip of the third molar tooth in the present study.

Researchers studying on plaster models have taken maxillary impressions at specific intervals as determined in their study design (166,210). Subsequently, dental stone casts were made out of the impressions by plaster material with low coefficient of expansion factor (166).

Some researchers have studied the orthodontic tooth movement on rats clinically on plaster models (134,166,187-89,210,213,215,230,277,293), and some investigators have assessed the amount of tooth movement with intraoral measurements (127,131,172-73,180,256 270, 280, 292) or on micro CT measurements (244,250,275,281).

Since orthodontic tooth movement is closely related with resorptive activity, the duration of our study investigating the effects of decortication amount on orthodontic tooth movement was determined as 21 days.

The remodelling cycle in rats varies between 10 to 31 days (300,301). There are researchers who advocate that the remodelling cycle is at least 6 days (299) as much as are there authors claiming that the span is dependent on age and is approximately 21 days for 6-month old rats (298). Baron (301) studied the relationship between the remodelling rate and the age in rats. The author investigated the trabecular remodeling of rat vertebrae in the tail and revealed that new bone formation rate in young adult rats (8 weeks) was 5 times greater than that of mature rats (12 weeks).

According to Lee et al. (245) and Cheung et al. (304), regional acceleratory phenomenon was observed on day 21 following corticotomy- and osteotomy-assisted tooth movement in rats. Similarly, Yaffe et al. (243) stated that 21 days corresponded to maximum resorption and regional acceleratory phenomenon in rats, therefore the duration of the experiment was determined as so.

Ren et al. (292) suggested that the duration of the experiments, which intend to clarify the characteristics of linear phase of tooth movement and evaluate biological reaction in this phase, should be at least 2 weeks.

Wilcko brothers claimed that the factor accelerating tooth movement is based on *regional acceleratory phenomenon,* which is characterized by increased bone turnover rate and decreased regional bone density in both soft and hard tissues (126, 248).

The studies investigating the duration of RAP in humans pointed out that RAP was initiated 1-2 days following corticotomy and this effect lasted up to 4 months (126, 253, 248). This period is known to be shorter in rats. Yaffe et al. (242) monitored RAP in

rats on day 10 after the intervention. In the same research, it was stated that most of the resorptive activity occurred during the first week. Tsai et al. (281) reported that RAP was observed within 2 weeks following micro-osteoperforation and corticotomy in their 6-week study in rats. Researchers monitored that orthodontic tooth movement rate was highest at the first week and decreased afterwards. During their 2-month observation, Lee et al. (245) spotted that resorptive activity occurred in the first week and reached a peak on day 21. Baloul et al. (251) has revealed that orthodontic tooth movement showed significant increase in rats only during the first week after selective alveolar decortication. Furthermore, they noted that osteoclastic activity lasted for 21 days. In conjunction with these results, Kim et al. (219) demonstrated that the tooth movement was predominantly within the first two weeks after corticision. After 14 days of flapless corticision-assisted orthodontic tooth movement, Murphy et al. (276) observed significant difference in the first week in the experiment group in terms of OTM amount, osteoclast and odontoclast numbers. However, the difference in these parameters diminished at day 14.

Sebaun et al. (236) assessed alveolar bone reaction as a result of selective alveolar decortication without applying orthodontic force and observed that the highest anabolic and catabolic activity was presented at the third week following surgery.

In our study, we used stereological investigation to evaluate histological changes based upon quantitative values. This investigation allows three-dimensional interpretation of two-dimensional cross sections of materials or tissues. Stereology method, that is becoming widespread thanks to the new generation computer programs and microscopes developed with the advancing technology, has brought objective quantitative parameters to the qualitative researches. Based on the principles of accuracy and objectivity, the stereological method has been preferred in this study in terms of being quantitative and reproducible.

In a research investigating the effects of local injection of platelet-rich plasma on rat orthodontic tooth movement, stereology method was used to evaluate alveolar bone density and bone volume similar to our study (222). Likewise, Erdem (279) has also used stereological investigation to asses alveolar bone volume parameters in her thesis study evaluating the clinical and histomorphometric effects of laser-asssited decorticotomy on rats.

In the present study, no statistically significant difference was found between the control group and experiment groups in terms of orthodontic tooth movement amount for 21 days. One possible reason for this result may be the magnitude of the force applied.

50 g of force might have been excessive for rat molars leading to compression and hyalinization of the periodontal ligament and thus, occurence of lag phase. However, we can not totally adress the results to presence of hyalinized areas, since bone at the compression side is not studied in the current research.

Osteoperforation studies in the literature vary according to the number and localization of the perforations as well as including flap elevation and the magnitude of the orthodontic force.

Kraiwattanapong and Samruajbenjakun (332) investigated the effects of light (LF) and heavy forces (HF) together with corticotomy on tooth movement rate, alveolar bone response, and root resorption in a rat model. Maxillary 1st molars were mesially moved by using a nickel- titanium closed-coil spring delivering either 10 g (light force) or 50 g (heavy force). For the corticotomy procedure, full-thickness flaps were elevated on the buccal and palatal sides, and two decortication marks were made on both the buccal and palatal cortical layers by using 0.5-mm round carbide bur. The depth of the decortication marks were adjusted to be half of the bur diameter. Tooth movement and alveolar bone response were assessed by micro–computed tomography (micro-CT) at day 0 as the baseline and on days 7, 14, 21, and 28. It was stated that the OTM amount in the HF group was significantly greater than in the LF group on days 7 and 14. However, no significant difference was found between the two groups at days 21 and 28. In terms of BV/TV ratio, there was no significant difference between the LF and the HF groups at all observed time periods. Similarly, no significant difference was detected between the groups regarding root resorption during the experiment period.

Tsai et al. (281) investigated the effects of flapless micro-osteoperforation and corticision on the rate of orthodontic tooth movement in 45 rats. The rats were divided into 3 groups, which each had 15 rats, as follows: micro-osteoperforation and orthodontic force (MOP + F), corticision and orthodontic force (C + F), and orthodontic force only (F, control). The force magnitude used to mesialize upper 1st molars was 50 g, which is same as our study. In the MOP + F group, three microperforations (0.25 mm in diameter and 0.25 mm in depth) were made 5 mm mesial to the 1st molar by using a round bur under low-speed handpiece. In the corticision group, the hammer was gently pushed through the gingiva until it reached the cancellous bone. When the rats were under anesthesia, OTM was assessed by weekly measurements of the distance between left maxillary incisor and left maxillary first molar using a digital ruler. Similar to our

study, the authors also observed no significant difference in the amount of OTM among the studied groups during 6 weeks, although significant difference was reported between experiment and control groups only at week 2. No significant difference observed in OTM amount among groups, except week 2, was attributed to the magnitude of the force applied. The authors suggested that force magnitude of 50 g might possibly induced a lag phase.

The role of hyalinization on orthodontic tooth movement was previously studied in the literature but not clarified yet. Even if the general assumption indicates that hyalinization, defined as cell-free compressed areas within the pressure site, occurrs under high force levels; it was stated that even with a force as low as 5 cN, hyalinization occurred and the timing of the event seemed to be independent of the force level (333). It was also stated that was that an initially light and gradually increasing force resulted in less hyalinization than a heavier initial force that increased to the same end force level (330). Furthermore, Iino et al. (244) stated that hyalinization of the PDL after corticotomy was observed only after 7 days, but not at later stages in beagle dogs. On the other hand, Böhl et al. (76) reported that hyalinization appeared not only in the initial phase of OTM, but also in the following stages. They noted that small hyalinized patches were observed in the later stages of orthodontic tooth movement. Compared to other species, the hyalinization was reported to appear in earlier phases of tooth movement in rats and mice. Higher alveolar bone densities of rats and narrower width of PDL, inducing higher forces and relatively more local strain in the alveolar bone leading to diminish in blood flow, can explain faster hyalinization. The narrow PDL space may end up with higher sensitivity of PDL to minor force level changes. Thus, reduction of the force level used in the current study might have created significant difference in OTM by surpassing the lag phase, which had possibly occurred.

There are also studies in the literature which conclude that tooth movement was not accelerated by alveolar decortication procedure, although they use less than 50 g of force.

Peron et al. (311) compared the histological responses in corticotomy- and corticision-assisted tooth movement in 90 rats. The animals were categorized into three groups, each having 30 rats, as follows: C (control—tooth movement only), CT (tooth movement + corticotomy), and CI (tooth movement + corticision). Upper 1st molars were

mesially displaced by 30 g of force. In the corticotomy group, after folding of the flap, animals received three perforations in the vestibular bone palate and two in the palatal bone plate of the first molar. The size of the perforations corresponded to bur diameter (0.25 mm). The cortical bone was perforated to reach the medullary bone. In the corticision group, the surgical hammer was inserted transmucosally penetrating the gingiva, cortical and cancellous bone. OTM was assessed intraorally by using a digital caliper. The distance measured was between the most mesial point of the first molar and the central incisor. The rats were sacrificed on days 3, 14 and 28. At the end of the experiment, authors found that osteoclast numbers in CT group were significantly higher than that of control group and observed less hyalinization areas in the corticision group compared to control and corticotomy groups on day 3. However, no statistically significant difference was found in terms of the tooth movement rate among the studied groups at different time points. The authors emphasized the necessity of shorter activation periods to increase osteoclast numbers, and thus, to enhance tooth movement.

The current findings of this study agree with that of Swapp et al. (313), who reported no significant difference in tooth movement amount after flapless awl-induced bone damage in the foxhounds. The authors claimed that one attributable reason for this may be the lack of separation of the periosteum in their study and suggested that more generalized damage, as in periosteal flap reflection, might be required to trigger osteocyte death and thus modelling. However, Librizzi et. al. (310) compared corticotomy and corticision groups, with and without mucoperiosteal flaps, on 60 male Wistar rats. The rats were divided into 5 groups, which were set to be at least 11 rats in a group. In their experiment, orthodontic tooth movement was made under 10-15 g force for 21 days. The corticotomy procedure consisted of reflecting a mucoperiosteal flap and making 3 osteoperforations located 5 mm mesial to the first molar using slow-speed handpiece. The size of the shallow perforations were adjusted to 0.25 mm of width and depth. By using a software, OTM was evaluated on digital photographs taken from the stone models. To assess OTM amount, the distance between distal groove of the 1st molar and mesial groove of the 2nd molar was measured. At the end of their study, the authors revealed that there was no significant difference in the amount of OTM between control and experiment groups, which is in aggreement with the current findings of this study. In order to make difference in OTM or alveolar response, they suggested that the surgical procedure might require more extensive injury including damaging the trabecular bone

beneath the cortical layer as in Kim et al.'s study (263), in which flappless corticision procedure was performed by inserting the blade 10 mm into the bone, providing further damage to underlying trabecular bone, in a feline model. In their study, orthodontic force of 100 g was used to retract canines of 16 cats for 28 days and it was stated that corticision is an effective procedure to accelerate tooth movement.

Similar to our study, Kurohama et al. (297) investigated the effect of bone volume cut, which is removed during corticotomy, on orthodontic tooth movement, root resorption and alveolar bone resorption. Total of 56 rats were used in the study. Following full-thickness flap elevation, two different orthodontic forces (10 gr and 25 gr) were applied for upper 1st molar mesialization and two different time periods were designed. In experiment group 1; 25 g of force was applied to mesialize upper first molars for 14 days and volumes of the bone cut by corticotomy were 0.1, 1.0, and 1.7mm^3. In experiment group 2; upper first molars were moved by 10 g of force for 14 days and volumes of the bone cut by corticotomy were 1.0 and 1.7mm^3. The coil springs were readjusted every 7 days. OTM was evaluated on 3D micro-CT images. The closest distance between the distal surface of the maxillary first molar and the mesial surface of the second molar was measured. As a result of their study, the comparison of OTM amount showed no significant difference among corticotomy and control groups in either experiment group 1 or 2. They also stated that no reactions specific to RAP was observed in the periodontal tissue of the animals. In terms of total root resorption area, there was no significant difference among any of the groups. The volume of resorbed bone around the moving tooth had increased proportionally with the volume of the bone cut in both Experiments 1 and 2. The authors concluded that corticotomy had no effect on tooth movement but it may increase alveolar bone loss after tooth movement, particularly reduced height of the alveolar bone. Furthermore, they noted that since articles, which indicate that tooth movement is accelerated by corticotomy, are more likely to be published in competitive academic environment; articles reporting negative results are in the minority in the literature.

One other explanation for the present results of this study may be the insufficiency of decortication marks, and thus trauma. It has been documented that the degree of the surgical insult to stimulate the bone is closely related with regional acceleratory phenomenon; that is the greater the stimuli, the larger the RAP (58,126). On the other hand, Kurohama et al. (297) evaluated the effects of different amount of bone removal

during corticotomy on orthodontic tooth movement and found no significant difference in orthodontic tooth movement amount between control group and corticotomy groups.

When the amount of tooth movements in control and experimental groups are compared, the results of this study are parallel with those of Librizzi et al.'s (310). Despite the difference in force magnitudes used in these studies, the tooth movement amounts are similar. However, orthodontic tooth movement amount established in the studies of Peron et al. (311) and Tsai et al. (281) are slightly above ours. One suggestable argument for this result could be the localization of the perforations. In the present study, the localization of the decortications was designed as to be apart from each other, that is on the alveolar crest, mesial and palatinal sides. In the other studies (281,311), perforations were performed to be in close vicinity, that is 1mm apart from each other. This proximity between the perforations might have enhanced the effectiveness of the surgical insult and thus, orthodontic tooth movement amount. However, further investigations are required for evidence proved arguments.

On the contrary to our findings, Cheung et al. (304) claimed that orthodontic tooth movement was accelerated by means of flapless micro-osteoperforation at the end of 21 days. One plausible explanation for this may be the number of osteoperforations. In this study, the authors preferred making 5 decortication marks, instead of 3 marks as done in our study. Even though RAP is suggested to be affected proportionally by the degree of trauma, the ineffectiveness of the degree of corticotomy amount on orthodontic tooth movement is among the evidences (297). Also, 0.8 mm depth of MOP in this study was not sufficient to evoke RAP response. Kim et al. (263) has shown that flapless corticision group in cats represented extensive direct resorption and rapid removal of hyalinised tissue which creates suitable environment for accelerated tooth movement. Yet, in that study, the cancellous bone was intentionally affected by the surgical blade, which may have triggered RAP.

The results of a previous animal study by Baloul et al. (251) illustrated that selective alveolar decortication, which was performed following full thickness flap elevation, affected orthodontic tooth movement rate only during the initial tooth placement phase, particularly at day 7. The tooth movement amount of the experiment group in the mentioned study was slightly lower than 1mm, which is parallel to our study; but the values of the control group differs from our study. When the control groups are compared, the average tooth movement of the control group of the present study is almost 5 times of that of Baloul et al.'s study. The reason in this difference might be attributed

to force magnitudes. 50 gram-force might have resulted in greater tooth movement in our study.

Another contradictive result was presented by Teixeira et al. (282), claiming that orthodontic tooth movement was accelerated twice in the micro-osteoperforation group at the end of 28 days. Even though the study design of their study is similar to the present study, including force magnitude and number of the perforations, the amount of tooth movement obtained quite differs. In the present study, orthodontic tooth movement amount in the control group was found to be almost 3.5 times that of the Teixeira et al.'s study. Furthermore, the tooth movement amount in our 3D group was almost 2 times that of their MOP group. The reason for the difference in OTM amounts in the present study and Teixeira et al.'s study can not be clarified due to absence of data regarding the measuring details (method, measuring points) in their published article. Similarly, as in the current research, Librizzi et al. (310) also mentioned that OTM amount in the control and experiment groups were different from those of Teixeira et al.'s. They attributed this result to the difference in the ages of the rats. Although the age of the rats used in the present study is parallel with those of Teixeira et al.'s; contradictively, our OTM results are parallel with those of Librizzi et al.'s.

In fact, these conflicting results underline the necessity of further investigations in corticotomy-assisted tooth movement studies in rats.

In order to clarify the tissue response, osteoclast numbers and the bone area between upper first and second molars were evaluated. Osteoclasts, indicating bone resorption, were evaluated histologically and bone parameters were evaluated histomorphometrically.

Despite the similar amount of OTM among groups, analysis of the histological sections revealed that osteoclast numbers on the mesial side of the upper 1st molar showed statistical increase in both 1D and 3D groups on day 14 and 21 compared to day 7. However, no significant difference was observed in the control group throughout the experiment period. Mesial osteoclasts also showed significant increase on day 7, 14 and 21 between groups. While no significant difference was observed between experiment and control groups on day 7, the osteoclast numbers in both experiment groups exhibited significant increase compared to control group on day 14. Nonetheless, on day 21, the significant increase was only observed in 3D group compared to the control group. The osteoclast numbers in 3D group were always increased significantly compared to 1D group at different time intervals.

Significant increase marked between control and experiment groups regarding osteoclast numbers on different days was not reflected to bone parameters. It is possible that three decortications might have stimulated osteoclast recruitment more, but still, osteoclast numbers in both experiment groups were not sufficient to accelerate tooth movement throughout the experiment period.

Histomorphometrical analysis was performed to analyse alveolar bone structure and its response. The change observed in osteoclast numbers was not totally reflected to histomorphometrical parameters. The difference between the groups became significant only on day 21. On day 21, BV/TV ratio, BVF were significantly increased and TVF was significantly decreased in control group compared to 1D group. Moreover, in the control group, BV/TV ratio and BVF was increased significantly on day 21 compared to day 7 and day 14. Oppositely, TVF in the control group decreased on the mentioned days.

Histomorphometrical parameters showed no significant difference in 1D and 3D groups during the experiment period. At the same time, no significant difference was present in 3D group on day 21 in any of the parameters. The reason for this might be the insufficiency of the sample size. It is considered that significant difference would have been observed in all histomorphometric values of 3D group on day 21 compared to control group, if larger sample size was obtained.

No significant increase was observed in the numbers of osteoclasts in the control group throughout the experiment period. This indicates that bone resorption was maintained in minimum levels and thus BV/TV ratio and BVF parameters were increased. Supporting this, the significant increase in osteoclast numbers in 1D and 3D groups reveals no increase in BVF and BV/TV ratio parameters.

Every change in BVF or increase in osteoclast numbers may not alter OTM amount. As shown by Kurohama et al. (297), who removed different amounts of bone with corticotomy, BVF was found to be inversely proportional with alveolar bone cut in 10-week old rats. However, the study indicated no increase in OTM magnitude as a reflection of this change. In agreement with the current findings, Tsai et al. (281) also reported no difference in BVF between flapless MOP, corticision groups and control groups at the end of three weeks. However, they reported increased number of osteoclasts in the experiment groups. Similarly, Librizzi et al. (310) found no difference in BVF nor osteoclast numbers between tooth movement and surgery groups after 21 days. As a result of their histomorphometrical analysis, Murphy et al. (276) also demonstrated that two different magnitudes of applied force with and without corticision had no efffect on

BVF and osteoclast numbers after 14 days of orthodontic tooth movement. As a matter of fact, one common point of the mentioned studies is the lack of surgical trauma in order to perform minimally invasive surgical procedure. On the contrary, Baloul et al. (251) performed intramedullary decortication marks, which were fully immersed into the bone, and stated that, during the inital 7 days, BV/TV ratio was significantly reduced while osteoclastic activity was increased. Also, Teixeixera et al. (282) demonstrated that osteoclast numbers increased while BVF decreased after 28 days of OTM with selective alveolar decortication on rats.

Increase in osteoclast numbers might have been associated with decrease in BVF, but since we wanted to evaluate new bone formation, the area investigated histologically in this study was limited between upper 1st and 2nd molars. It may be logical to evaluate upper 1st molar circumferentially in order to detect generalized decrease in bone density parameters. This may be another limitation of this study.

In orthodontic tooth movement, bone resorption is seen in the compression zone, which is at the same direction as the tooth movement, whereas new bone is formed in the tension zone, which is in the opposite direction of tooth movement. While the major cells in bone resorption are osteoclasts, osteoblasts are responsible for new bone formation. Even though osteogenesis was stated to occur after 14 days (323), in our study, no significant increase in alveolar bone parameters indicating new bone formation was observed at the area located between upper 1st and 2nd molars in single and three decortication groups. One reason for this may be the insufficiency of experiment duration to clearly observe the bone formation phase.

In bone remodeling phases, new bone formation occurs after resorption phase, following the drop in osteoclast numbers. Since 21 days correspond to maximum resorption in rats, it can be concluded that the experiment period needs to be extended to monitor new bone formation after the resorption phase.

Reddi et al. (315) transplanted bone powder subcutaneously to 25-35-day old rats and demonstrated that the first bone was visible on day 10, while larger masses of bone was observed on days 18-21. Baron et al. (317) claimed that total duration of the remodeling sequence in the alveolar bone of the adult rat is 10-15 days. However, the ages of the rats were not specified. Milne et al. (318) applied buccally directed force to rat molars via archwires and noted that secretory osteoblasts were seen on day 7 in 6-week-old rats. Taking into account the fact that the animals used in our study are 16 weeks old, we can conclude that the test period has to be extended to monitor the bone formation.

On the other hand, the duration of the experiments performed on other animals (cats, dogs, rabbits) to monitor bone formation varies between 1-3 months in the literature (318-21).

Alveolar bone response can change according to intervention. In a study investigating the alveolar bone response after decortication in rabbits, at the end of 14 days, it was manifested that orthodontic tooth movement was accelerated and osteoclast numbers were increased in the experiment group. No new bone formation was noted in both groups. These results support the hypothesis that decortication evokes resorptive response rather than new bone formation. However, Sebaun (236) evaluated alveolar response in rats after selective alveolar decortication and concluded that at week 3, the surgery group had significantly higher osteoclast number, and greater lamina dura apposition width. They also noted that the catabolic activity (osteoclast count) and anabolic activity (apposition rate) were three-fold greater than control group. On the other hand, lack of new bone formation might support the hypothesis that decortication stimulates bone resorption rather than new bone formation.

6. CONCLUSION

- Transmucosal decortication procedure performed with slow handpiece increased the osteoclast numbers on the distal and especially on the mesial sides of the upper 1st molar, which was orthodontically moved. Single perforation in bone also increased osteoclast numbers.

- No significant difference was observed in terms of orthodontic tooth movement amount between the studied groups during 21 days.

- In the area located between the upper 1st and 2nd molars, the ratio of bone volume over total tissue decreased in 1D group on day 21. However, it is considered that if the sample size of the current study was larger, a significant difference in this ratio might have been observed also in 3D group on day 21.

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YEDİTEPE ÜNİVERSİTESİ

T.C. YEDİTEPE ÜNİVERSİTESİ, DENEY HAYVANLARI ETİK KURULU

(YÜDHEK)

ETİK KURUL KARARI

Toplantı Tarihi	Karar No	İlgi	Proje Yürütücüsü
11.01.2016	500	23.12.2015	Prof.Dr.Fulya ÖZDEMİR

'Sıçanlarda Alveolar Dekortikasyon Sayısının Diş Hareketine Olan Etkilerinin Klinik Ve Histomorfometrik Olarak İncelenmesi' adlı bilimsel çalışma etik kurulumuzda görüşülmüş olup, çalışmanın etik kurallara uygun olduğuna oy birliğiyle karar verilmiştir.

Etik Onay Geçerlilik Süresi: 1 Yıl	Hayvan Türü ve cinsiyeti: Sıçan ♂	Hayvan Sayısı: 72

GÖREVİ	ADI SOYADI	İMZA
Başkan	Prof. Dr. M. Ece GENÇ	KATILMADI
Başkan Yardımcısı	Prof. Dr. Erdem YEŞİLADA	
Raportör	Prof. Dr. Işıl Aksan KURNAZ	
Üye	Prof. Dr. Bayram YILMAZ	
Üye	Prof. Dr. Başar ATALAY	
Üye	Doç. Dr. Soner DOĞAN	
Üye	Yard. Doç. Dr. Ediz DENİZ	
Üye	Doç. Dr. C. Narter YEŞİLDAĞLAR	KATILMADI
Üye	Sumru KİRAZCI	

EK 13. Özgeçmiş

Kişisel Bilgiler

Adı	BEGÜM	**Soyadı**	ASLAN
Doğum Yeri	ÜSKÜDAR	**Doğum Tarihi**	04.04.1990
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Öğrenim Durumu

Derece	Alan	Mezun Olduğu Kurumun Adı	Mezuniyet Yılı
Doktora	ORTODONTİ	**YEDİTEPE ÜNİVERSİTESİ**	2018
Yüksek Lisans	-		
Lisans	DİŞ HEKİMLİĞİ	**MARMARA ÜNİVERSİTESİ**	2011
Lise	-	**MERSİN FEN LİSESİ**	2006

[#] Başarılmış birden fazla sınav varsa(KPDS, ÜDS, TOEFL; EELTS vs), tüm sonuçlar yazılmalıdır

Bildiği Yabancı Dilleri	Yabancı Dil Sınav Notu (#)
İNGİLİZCE	IELTS (8.0), ÜDS (93)

İş Deneyimi (Sondan geçmişe doğru sıralayın)

Görevi	1.1.1 Kurum	Süre (Yıl - Yıl)
		-
		-

Bilgisayar Bilgisi

Program	Kullanma becerisi
MICROSOFT OFFICE	İYİ

*Çok iyi, iyi, orta, zayıf olarak değerlendirin

Bilimsel Çalışmaları

SCI, SSCI, AHCI indekslerine giren dergilerde yayınlanan makaleler

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-

Diğer dergilerde yayınlanan makaleler

-
-

Uluslararası bilimsel toplantılarda sunulan ve bildiri kitabında (*Proceedings*) basılan bildiriler

Hakemli konferans/sempozyumların bildiri kitaplarında yer alan yayınlar

Diğer (Görev Aldığı Projeler/Sertifikaları/Ödülleri)
