Insulin like growth factor-I (IGF-I), its receptor (IGF-IR) and Insulin receptor substrate 1 (IRS1) expression as an early reaction of PDL cells to experimental tooth movement in the rat

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Hohen Medizinischen Fakultät
Rheinische Friedrich-Wilhelms-Universität
Bonn

Yasser Kheralla

aus

Aleppo/ Syrien

2013

Anfertigung mit Genehmigung der
Medizinischen Fakultät der Universität Bonn

1. Gutachter: Prof. Dr. Werner Götz

2. Gutachter: Prof. Dr. Stephan Baader

Tag der Mündlichen Prüfung: 22.05.2013

Aus der Poliklinik für Kieferorthopädie des Zentrums für Zahn-, Mund-, und Kieferheilkunde der Universität Bonn
Direktor: Professor Dr. A. Jäger
Labor für Oralbiologische Grundlagenforschung
Leiter: Professor Dr. Werner Götz

Meinen Eltern
meiner Frau
und meinen Kindern
in Dankbarkeit gewidmet

Inhaltsverzeichnis

1. Zusammenfassung

1.1 Einleitung

Die Applikation einer Kraft auf einen Zahn im Rahmen einer kieferorthopädischen induzierten Zahnbewegung führt zu einer initialen Auslenkung des Zahnes innerhalb seiner Alveole, sodass einige Teile des Desmodontalspaltes sich verschmälern („Druckzone“) und gegenüberliegenden Teile sich verbreitern („Zugzone“).

Aufgrund von Zirkulationsveränderungen, Zelldeformation, oder Kompression der Nervenenden erfolgt eine Freisetzung verschiedener Faktoren, die eine Resorption des Alveolarknochens auf der Druckzone und eine Knochenapposition auf der Zugzone verursachen.

IGFs stellen eine Familie von Wachstumsfaktoren dar, die aus 2 Liganden, IGF-I/II, 2 Rezeptoren, IGF-IR/IIR, und 6 Bindungsproteinen, IGFBP-1 bis IGFBP-6, bestehen. IGF-I stellt einen wichtigen Faktor für postnatales Wachstum dar. Er wirkt sowohl endokrin als auch para- und autokrin auf systemischer als auch auf lokaler Ebene. Im Bindegewebe und im Knochen wird IGF-I von Osteoblasten und Fibroblasten produziert und in die Matrix eingelagert. Es stimuliert Differenzierung und Proliferation und hemmt die Apoptose dieser Zellen. Nach Bindung von IGF-I an IGF-IR werden 3 intrazelluläre Signalwege aktiviert. IRS-1 ist dabei das wichtigste Substrat für die Vermittlung der IGF-I-Signale.

Knochen und Bindegewebe empfangen mechanische Belastungen, die für ihre Integrität und Ihr Remodelling von Bedeutung sind, was zur Freisetzung verschiedener Faktoren führt. In vielen Geweben und Zellarten induziert die mechanische Belastung auch eine IGF-I-Expression. In neuen Studien konnten alle Moleküle des IGF-Systems im Parodont des Menschen und der Ratte nachgewiesen werden. Des Weiteren konnten Studien zeigen, dass Dehnungen der Zellen eine Modulation der Genexpression verschiedener IGF-Familienmitglieder zur Folge hatten.

1.2 Zielsetzungen und Hypothesen der vorliegenden Studie

In der vorliegenden Untersuchung sollte überprüft werden:

1. ob lokal produziertes IGF-I an der Mechanotransduktion von Zellen des Parodontalligaments (PDL) während der frühen Phase der Zahnbewegung beteiligt ist,

2. ob es ein differenziertes Expressionsmuster von IGF-I, IGF-IR und IRS1 in Druck- und Zugzonen gibt,

3. und ob die Veränderungen bzw. die Verteilung des IGF-I von der Größe der angewandten Kräfte abhängig sind.

Darüberhinaus sollte die Bedeutung von IGF-I für die Umbauaktivitäten im Rahmen der orthodontischen Zahnbewegung diskutiert werden.

1.3 Material und Methoden

Der rechte obere erste Molar von 20 anästhetisierten Ratten wurde mit einer Kraft belastet, um den Zahn nach mesial zu bewegen. Konstante Kräfte von 0.1N, 0.25 N und 0.5 N wurden über 4 Stunden an 4 Versuchstieren verwendet. Die Kräfte wurden über einen Sensor für 4 Stunden gemessen. Weiterhin wurden konstante Kräfte von 0.1 N über 2 Stunden in 8 weiteren Tieren angewandt. Im Anschluss wurden der erste und zweite Molar permanent mit Kunststoff separiert und die antagonistischen Molaren beschliffen. Vier Ratten aus der Gruppe wurden nach 1 Tag und weitere 4 Ratten nach 2 Tagen geopfert. Die unbehandelte kontralaterale Seite wurde als Kontrolle verwendet. Die präparierten Maxillae wurden in Paraffin eingebettet und schließlich in dünne sagittale Schnitte geschnitten. Immunohistologische Färbungen wurden zur Identifizierung von IGF-I, IGF-IR und IRS1 durchgeführt. Fotos wurden von der Druckseite (mesiokoronale Region) und von der Zugseite (distokoronale Region) aufgenommen und histomorphometrisch ausgewertet. Die statistische Auswertung erfolgte mit SPSS-Software.

1.4 Ergebnisse und Diskussion

Alle untersuchten Faktoren waren im PDL der belasteten Gruppen und der Kontrollgruppe nachweisbar. Die Ergebnisse zeigten eine Zunahme der Anzahl der positiven Zellen aller untersuchten Faktoren auf der Zugzone. Im Vergleich mit der Kontrollgruppe waren alle Unterschiede signifikant und von der Zeitdauer der Belastung abhängig. Die höchste Zahl der IGF-IR-immunopositiven Zellen wurde nach 24 Stunden von der Kraftapplikation festgestellt.

In der Druckzone fiel die Anzahl der positiven Zellen aller untersuchten Faktoren nach 4 Stunden von der Kraftapplikation ab. Diese Abnahme war signifikant von der Höhe der applizierten Kräfte abhängig. Nach 24 bzw. 48 Stunden stieg die Anzahl der positiven Zellen wieder auf den Level der Kontrollgruppe an.

Die Abnahme/Zunahme der positiven Zellen war tendenziell von der Kraftgröße abhängig.

Diese Ergebnisse weisen auf eine Rolle von IGF-I in der Aufrechterhaltung der parodontalen Gewebshomöostase sowie den Umbauprozessen während der Zahnbewegung hin. Die Ergebnisse in der Zugzone stimmen mit denen anderer Autoren überein, die für andere Gewebe gezeigt haben, dass nach einer kurzfristigen Belastung die IGF-I-Expression in Sehnenzellen, Muskelzellen aber auch in Osteoblasten und Osteozyten zunimmt.

Man kann auch davon ausgehen, dass IGF-I an Mechanotransduktionsvorgängen während der frühen Phasen der Zahnbewegung beteiligt ist.

Diese IGF-I-Hochregulation könnte die Proliferation und die Differenzierung der PDL-Zellen stimulieren und die Apoptose beeinflussen.

In der Druckzone zeigen die Ergebnisse, dass verminderte Expression der untersuchten Faktoren zur Verringerung der Proliferation und schließlich zur Abnahme der Anzahl der PDL-Zellen führen könnte.

1.5 Schlussfolgerung

In der vorliegenden Studie wurde in einem Rattenmodell die Verteilung von IGF-I, IGF-IR und IRS1 während der frühen Phase der Zahnbewegung untersucht.

Die erhobenen Daten deuten darauf hin, dass die lokale auto- und parakine Freisetzung von IGF-I eine frühe Reaktion der Zellen des PDLs auf eine orthodontische Belastung darstellt, und dass IGF-I an Mechanotransduktionsvorgängen beteiligt ist. Diese Reaktion ist tendenziell von der Größe der applizierten Kraft und eindeutig von Belastungszeit abhängig. Dies könnte zur Aktivierung bzw. Hemmung der Proliferation und Differenzierung der PDL-Zellen sowie zur Hemmung bzw. Auslösung von Apoptose führen.

Die Ergebnisse dieser Studie tragen dazu bei, die Molekulare und zelluläre Grundlagen der kieferorthopädischen Zahnbewegung besser zu verstehen und die mit den klinischen Erfahrungen aus der Kieferorthopädie zu verknüpfen.

2. Introduction

2.1 Tooth movement

Orthodontic tooth movement has been defined as the result of a biologic response of periodontal ligament to an externally applied force (Proffit, 2007).

The purpose of orthodontic treatment is to move teeth as efficiently as possible with minimal adverse effects for the teeth and supporting tissue. The predictability of tooth movement in humans is of clinical interest, particularly in terms of the nature and speed of tooth movement in response to specific applied mechanics and the absence of undesirable sequelae (Iwasaki et al., 2008).

Mainly based on histological research, a pressure and a tension side are distinguished during orthodontic tooth movement. On the pressure side, the biological events are as follows: disturbance of blood flow in the compressed PDL, cell death in the compressed area of the PDL (hyalinization), resorption of the hyalinized tissue by macrophages, and undermining bone resorption by osteoclasts beside the hyalinized tissue, which ultimately results in tooth movement. On the tension side, blood flow is activated where the PDL is stretched, which promotes osteoblastic activity and osteoid deposition, which later mineralizes (Von Böhl and Kuijpers-Jagtman, 2009).

2.1.1 The theories of tooth movement

2.1.1.1 The pressure-tension theory

According to the classic histological research by Sandstedt (1904), Oppenheim (1911) and Schwarz (1932), this theory states that a tooth moves in the periodontal space by generating a “pressure side” and a “tension side” (Krihnan and Davidovitch, 2006).

In this theory, the blood flow decreases where the PDL is compressed, and maintains or increases where the PDL is under tension. These alterations create quickly changes in chemical environment (Proffit, 2007).

The force-subjected PDL progenitor cells differentiate into compression associated osteoclasts and tension-associated osteoblasts, causing bone resorption and apposition, respectively. Direct resorption is associated with light force (frontal) application.

Indirect (undermining) resorption and hyalinization are associated with bio-intolerant heavy forces (Masella and Meister, 2006).

2.1.1.2.1 The bone-bending theory

According to Baumrind (1969) and Grimm (1972), this theory states that orthodontic forces produce alveolar bone deflection. The active biologic processes that follow bone bending involve bone turnover and renewal of cellular and inorganic fractions and are accompanied by changes in the PDL. These authors could explain facts such as (1) the relative slowness of en-masse tooth movement; (2) the velocity of tooth movement toward an extraction site; and (3) the relative rapidity of tooth movement in children, who have less heavily calcified and more flexible bones than adults. The increase of convexity at the pressure side is associated with bone resorption, whereas the increase of concavity at the tension side is associated with bone formation (Kirshnan and Davidovitch, 2006).

2.1.1.2.2 Piezoelectricity

Piezoelectricity is a phenomenon observed in many crystalline materials, in which a deformation of a crystal structure produces a flow of electric current (Kirshnan and Davidovitch, 2006). After bone bending, the distortion of crystalline structures of bone generates bioelectric charges and can regulate the remodeling of alveolar bone during orthodontic tooth movement. Concave bone surfaces characterized by osteoblastic activity are electronegative; convex bone surfaces characterized by osteoclastic activity are electropositive or electrically neutral (Meikle, 2006).

It should be noted that these piezoelectric signals have two unusual characteristics: (1) a quick decay rate and (2) the production of an equivalent signal, opposite in direction, when the force released, therefore the sustained orthodontic tooth movement does not produce prominent stress-generated signals (Proffit, 2007).

2.1.2 Phases of tooth movement

There are 4 phases of tooth movement. The first phase (initial phase) lasts 24 hours to 2 days, the cellular and tissue reactions start in this phase of tooth movement immediately after force application. Depending on the magnitude of applied force, the tooth movement stops in the second phase (lag phase) for 20 to 30 days because of the induced necrosis of cellular elements within the PDL at the pressure side. The phagocytic cells, which recruit from the adjacent marrow spaces and from the direction of the viable PDL, remove the necrotic tissues from compressed PDL sites and adjacent alveolar bone.

After the removal of necrotic tissue (hyalinization) formed during the second phase, tooth movement is accelerated in the third phase (postlag phase or acceleration stage) and continues into the fourth phase (linear stage) (Burstone, 1962; Pilon et al., 1996; Van Leeuwen et al., 1999, Kirshnan and Davidovitch, 2006). Proffit refers that heavy forces lead to undermining resorption while the lighter forces enable frontal resorption without delay (Proffit, 2007).

It must be kept in mind that hyalinization zones at the pressure areas could form even during third and fourth stage, especially in areas where high forces were applied (Von Böhl et al., 2004).

2.1.3 Optimal orthodontic force

Schwarz defined optimal continuous force as ‘‘the force leading to a change in tissue pressure that approximated the capillary vessels’ (Schwarz, 1932). Storey and Quinn correlated optimal force to the surface area of the root (Storey and Smith, 1952; Quinn and Yoshikawa, 1985).

Traditionally, orthodontic forces have been categorized as “light” or “heavy,” and it was assumed that light forces are preferable, because of their ability to evoke frontal resorption of bone and therefore more physiologic than heavy forces that cause necrosis (hyalinization) of the PDL and undermining bone resorption (Kirshnan and Davidovitch, 2006). Since some trauma is always associated with applied orthodontic forces, even light ones, and precise measurement of force applied to roots or parts

thereof is impossible, a current concept of optimum force was performed. This concept means that there is a force of certain magnitude and temporal characteristics (continuous versus intermitted, constant versus declining) capable of producing a maximal rate of tooth movement, without tissue damage, and with maximum patient comfort. The optimal force for tooth movement may differ for each tooth and for each individual patient (Ren et al., 2003).

These orthodontic forces deform the extracellular matrix and activate cells of the paradental tissues in sequential cellular and molecular events that lead to convert the mechanical energy to biochemical and/or electrical signals. This process is known as mechanotransduction (Krishnan and Davidovitch, 2009).

2.2 Mechanotransduction in bone and PDL

In nearly all aspects of biology, forces are a relevant regulator of life's form and function. More recently, science has established that cells are exquisitely sensitive to forces of varying magnitudes and time scales, and they convert mechanical stimuli into a chemical response. This phenomenon, termed mechanotransduction, is an integral part of cellular physiology and has a profound impact on the development of the organism. Furthermore, mal-functioning mechanical properties or mechanotransduction often leads to pathological alterations of the organism (Kolahi and Mofrad, 2010).

There are four essential interrelated steps in the transduction of mechanical signals by tissues: sensing the mechanical signal by the cells, transduction of this mechanical signal into biochemical one, transmission of the biochemical signal to the effector cells, and the effectors cell response (Wise and King, 2008).

Mechanosensing is a process by which cells sense structural changes in the extracellular matrix (ECM), caused by external mechanical loading. Each cellular system in paradental tissues is equipped with mechanosensors; thus, each mechanical stimulus may activate multiple mechanosensors, followed by downstream cellular events (Krishnan and Davidovitch, 2009).

In bone, bone cells respond to fluid flow stimulation *in vitro* by the production of mediators such as nitric oxide (NO) and prostaglandins (Klein-Nulend et al., 2005).
The signal might be transduced to the osteocytes through specific receptors or through deformation of the cytoskeleton (Duncan and Turner, 1995). The cell deformation may occur indirectly through fluid flow and/or directly through integrins (Hennemann et al., 2008).
Many studies suggest that the mechanosensory molecules are: ion channels, integrins, G-proteins, the Wnt surface receptor and the cytoskeleton (Sawakami et al., 2006; Scott et al., 2008).
Gated ion channels are involved in hearing and touch as well as maintaining osmotic homeostasis and regulating cell volume. In these contexts, tension created in the cytoskeleton in response to loading can alter the shape of the membrane lipid bilayer, resulting in changes in ion channel behavior (Hamill and Martinac, 2001; Jacobs et al., 2010). There is also evidence of interplay between different channels and between channels and other molecular mechanosensors. For example, annexin V is a calcium channel that binds extracellular collagen and cytoskeletal actin. It is involved in the intracellular calcium response to fluid flow in osteoblasts. This suggests the possibility that mechanical force in the actin cytoskeleton may open this channel (Haut Donahue et al., 2004, Jacobs et al., 2010).
Integrins, which are present on the cell membrane, connect the intracellular actin filaments through a variety of cytoskeletal linker proteins to ECM. This focal adhesion complex not only transduces matrix strain to the cytoskeleton but also activates protein kinases that initiate a variety of intracellular signalling pathways (Wang and Thampatty, 2006; Schwartz, 2011). There is cross-talk between integrins and growth factors receptors occurs at multiple levels. For example, integrin αvß3 enhances insulin-like growth factor-I receptor (IGF-IR) signaling through FAK-dependent phosphorylation of IGF-IR. Mice that lack this gene in embryonic fibroblasts showed impaired phosphorylation of IGF-IR in response to insulin-like growth factor-I (IGF-I), and IGF-I-dependent cell proliferation is blocked (Liu et al., 2008; Legate et al., 2009).

Guanine nucleotide–binding proteins or G-proteins are signaling GTPases that alternate between an active GTP-bound state and an inactive GDP-bound state. A G-protein-coupled receptor includes a transmembrane receptor and an intracellular G-protein, which is activated (GDP replaced with GTP) owing to a conformation change that occurs with ligand binding or mechanical stimulation (independent of ligand binding) (Chachisvilis et al., 2006; Jacobs et al., 2010).

The Wnt surface receptor, low-density lipoprotein receptor related protein 5 (LRP5), has been suggested as a key regulator of bone mass. Its signaling pathway may be important for the osteogenic response to loading (Sawakami et al., 2006; Wise and King, 2008).

The cytoskeleton represents a system of intracellular fibers and accounts for much of the structural integrity of the cell, incorporating microtubules, intermediate filaments and actin filaments. These networks are thought to maintain a constant, basal level of tension, so that small, local deformations affect overall cytoskeletal tension, initiating cellular responses (Myers et al., 2007).

Several *in vitro* studies demonstrated the involvement of extracellular and intracellular signaling components in mechanotransduction (Liedert et al., 2005). The earliest experiments showed that the immediate response of cells to mechanical strain was the generation of prostaglandins (PGs), nitric oxide (NO), the second messenger cyclic adenosine monophosphate (cAMP) and inositol phosphates. Changes in intracellular [Ca 2+] were shown to occur via stretch-activated ion channels (Harell et al., 1977; Yeh and Rodan, 1984; Sandy et al., 1989; Davidson et al, 1990; Klein-Nulend et al, 1995; McDonald et al., 1996; Meikle, 2006). Thus, the transduction of the mechanical signals into biochemical signals begins.

These messengers will evoke a nuclear response which will either result in production of factors responsible for osteoclast recruitment and activation, or bone forming growth factors (Roberts-Harry and Sandy 2004).

The downstream signalling pathways during mechanical stretching appear to overlap with known growth factor and hormone signals (Scott et al., 2008) (Fig. 1).

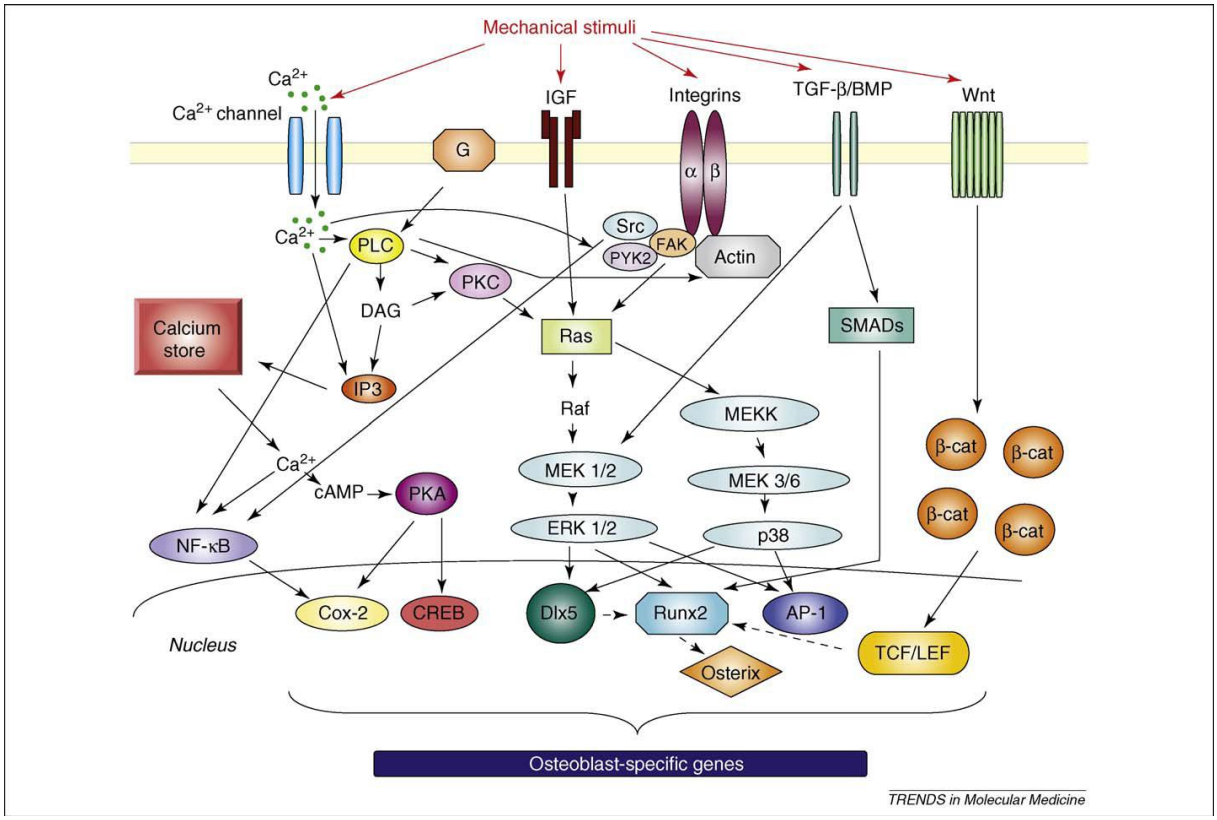

Figure 1: Schematic diagram of interactions of different signaling pathways during mechanical stretching. Membrane-bound receptors such as Ca^{2+} channels, integrins, Gproteins, IGF and TGF-b and/or BMP receptors are stimulated by mechanical forces, resulting in induction of several transcription factors that regulate osteoblast differentiation and formation. AP-1 and Runx2 are induced mainly through MAPK and SMADs pathways. Runx2 is also stimulated via the Wnt pathway, involving b-catenin and TCF or LEF factors. PLC–PKA pathway contributes to NF-kB, Cox-2 and CREB induction. Abbreviations: AP-1, activator protein-1; b-cat, b-catenin; DAG, diacylglycerol; FAK, focal adhesion kinase; G, G-protein; IP3, inositol (1,4,5)-trisphosphate; MEKK, MAPK kinase kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PYK2, proline-rich tyrosine kinase 2 (From Papachroni et al., 2009).

Discoveries in mechanobiology have illuminated sequential cellular and molecular events, such as synthesis and secretion of specific products (Krishnan and Davidovitch, 2009). One of these molecules is Insulin-like growth factors (IGFs).

2.3 Insulin-like growth factor peptide family

IGFs represent a family of endocrine, paracrine and autocrine polypeptides. The IGF family is comprised of ligands (IGF-I, IGF-II, and insulin), six well characterized binding proteins (IGFBP-1 though -6), and cell surface receptors that mediate the actions of the ligands (IGF-I receptor, insulin receptor, and the IGF-II mannose-6-

phosphate [(M-6-P) receptor] (LE Roith et al., 2001; Lelbach et al., 2005; Annunziata et al., 2001) (Fig. 2).

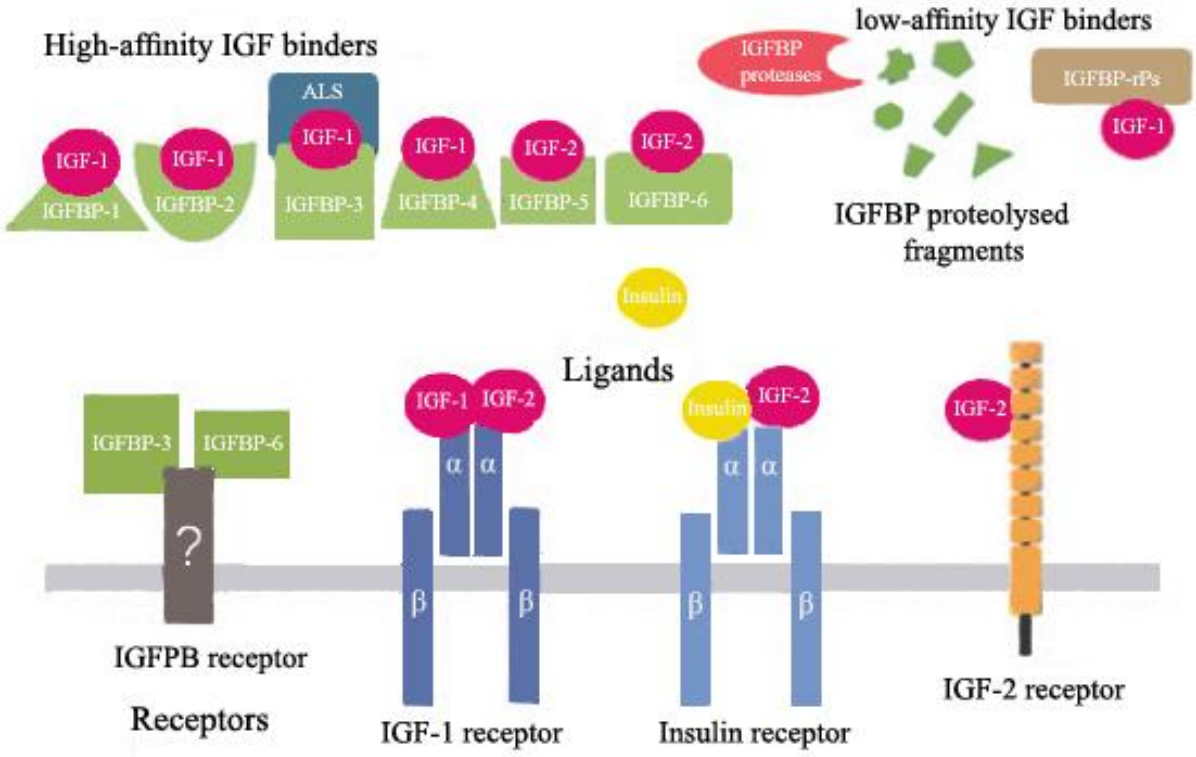

Figure 2: *The IGF family and related signaling molecules. Ligands, cell-surface receptors, and the IGF-binding proteins (IGFBPs) constituting the IGF system are shown. Molecules binding IGF with low affinity, including IGFBP-related proteins (IGFBP-rPs) and IGFBP fragments generated by IGFBP protease cleavage, are also indicated. IGF-1 interacts with IGF1R and IGFBPs, IGF-2 interacts with IGF1R, IGF2R, IR and IGFBPs, in particular IGFBP- 5 and -6. Insulin interacts with IR. Some IGFBPs, IGFBP-3 and –5 in particular, may exert their effects via their own receptor independently of their capacity to modulate IGF signaling through IGF1R or IR (from Dupont and Holznberger, 2003).*

2.3.2 Insulin-like growth factors

IGF-I is a trophic factor that circulates at high levels in the blood stream. Although the main source of IGF-I in the serum is liver, many other tissues synthesize it and are sensitive to its action, especially during postnatal development. Regulation of hepatic IGF-I production is mostly mediated by growth hormone (GH) and insulin. In turn, IGF-I feeds back to suppress growth hormone and insulin release (Pavelic et al., 2007).

The synthesis of IGF-I is controlled by a number of endogenous (genetic and hormonal) and exogenous (nutrition and physical activity) factors. About one-third to one-quarter of IGF-I expression is determined by exogenous factors (Zofkova, 2003).
The synthesis of IGF-II is relatively GH-independent. Its expression is much higher during fetal development than in postnatal life. It acts as a regulatory peptide; it is mitogenic for a number of cell types (Pavelic et al., 2007).
IGF-I and IGF-II are single-chain polypeptides, with sequences 62 % identical to that of proinsulin. However, unlike insulin and other peptide hormones, they are not produced and stored within the cells of a specific tissue, but may instead be produced by almost any cell in the body (Dupont and Holzenberger 2003).
The ligands IGF-I and IGF-II are involved in various cellular processes including differentiation, proliferation, morphogenesis, growth, apoptosis, control of metabolic function and carcinogenesis **(**Clemmons AND Maile 2005; Danley et al., 2005).

2.3.3 Insulin-like growth factor receptors

Most of the cellular effects of the IGFs are mediated by binding to the IGF-IR. The IGF-IR is a transmembrane tyrosine kinase structurally similar to the insulin receptor. It is composed of two extracellular α-subunits and two intracellular β-subunits (Laron, 2001). The α-subunits bind IGF-IR to IGF-I, IGF-II and insulin at supraphysiological concentration, while the β-subunits transmit the ligand-induced signals (Jones and Clemmons, 1995; Mauro and Surmacz, 2004). Binding of the ligands to the IGF-IR induces its autophosphorylation via tyrosine kinase activity. This, in turn, causes the interaction with certain cellular substrates such as insulin receptor substrate 1 (IRS1), adapter protein Shc and 14-3-3-proteins (Romano, 2003; Laviola et al., 2007).
A major intracellular substrate for the IGF-IR is IRS1 which, after its tyrosine phosphorylation, initiates multiple signallings. It mediates the transmission of mitogenic, metabolic and antiapoptotic signals (Jones and Clemmons, 1995; Ogata et al., 2000).
IGF-II receptor is a single large transmembrane receptor which bind with IGF-II and, with a much lower affinity, with IGF-I. The IGF-II receptor does not appear to act as a

traditional signaling receptor in response to IGF binding and is thought to act mainly as a clearance receptor for IGF-II (Holly and Perks, 2006; Samani et al., 2007).

2.3.4 IGF binding proteins and proteases

IGFBPs are a family of secreted proteins that bind IGF with high affinities that are greater than those of the IGF-IR. Six distinct IGFBPs, designated as IGFBP-1 to -6, have been isolated and characterized from human and a variety of vertebrate species (Firth and Baxter, 2002; Kelley et al., 2002).

IGFBPs function as carrier proteins for circulating IGFs and serve not only as a reservoir for IGFs release, but also greatly increases the half-life of IGFs, and regulate IGF turnover, transport of IGFs from the vascular space to target tissues, and tissue distribution. IGFBPs expressed in many peripheral tissues. They have been shown to inhibit or potentiate IGF actions *in vitro*. While IGFBP-4 and IGFBP-6 have been consistently found to inhibit IGF actions, IGFBP-1, -2, -3, and -5 can both inhibit and potentiate IGF actions, depending on cell type (Duan and Xu, 2005).

There is compelling evidence that some IGFBPs possess biological activities that are ligand-independent. These include growth inhibition, direct induction of apoptosis, and modulation of the effects of other non-IGF growth factors (Firth and Baxter, 2002; Mohan and Baylink, 2002). For example, IGFBP-3 inhibits cell growth in the absence of IGFs and in IGF-1R-null cells (Butt and Williams, 2001).

IGF-BP degrading proteases act as growth stimulators by increasing local IGF availability. They fall into three major categories. Kallikrein-like serine proteases, the second major category is cathepsins and intracellular proteinases, and the third category involves matrix metalloproteinases. Proteolytic activity by proteases may play a role in normal and abnormal tissue proliferation by cleaving IGF-BP into fragments with lower affinity for IGFs, thereby increasing the levels of free IGFs to activate IGF-IR (Cohen, 2006). These proteases are under the control of autocrine, paracrine, and hormonal influences (Rosen, 1999).

2.3.5 The role of IGFs in bone and oral biology

In bone, many factors regulate homeostasis. IGF-I and IGFII are the most abundant growth factors stored in bone and are locally produced by bone cells. IGFs increase bone formation by regulating proliferation, differentiation, and apoptosis of osteoblasts (Govoni et al., 2005).

Consistent with this role, patients with Laron syndrome, caused by IGF-I deficiency, present marked osteoporosis (Laron et al., 1998).

Genetic modifications in specific components of the IGF system have provided tremendous insights into the role of the IGFs and IGF-IR *in vivo*. For example, the overproduction of IGF-I in the osteoblasts of transgenic mice increases bone formation (Zhao et al., 2000), whereas the overproduction and deficiency of IGF-II in mice has no major effect on skeletal size or bone turnover in mice (DeChiara et al., 1991; Wolf et al., 1995). In addition, mice lacking IGF1R display marked organ hypoplasia and delayed skeletal ossification (Yakar and Rosen, 2003)

The IGFs fulfills an important role in growth and development of teeth, mandible, maxillae, and tongue. It has been postulated that IGF-I may be of great value in the treatment of periodontal defects and in tissue healing (Werner and Katz, 2004).

The suitability of IGF-I, in combination with other growth factors, for periodontal regeneration has been tested (Palioto et al., 2004, Sant'Ana et al., 2007).

It is believed, that IGFs behave as poliferative factors for cementoblasts (Grzesik and Narayanan, 2002). They are involved in early root formation by regulating the mitotic activity of the outer layer of the epithelial cells of Hertwig's root sheath (Fujiwara et al., 2005).

Recently, the distribution pattern of IGFs components was investigated in both deciduous and permanent teeth. Many of them were found to be localized in the periodontium of human and rats (Götz et al., 2001; 2003; 2006a).

2.3.6 IGF-I and mechanical loading

Mechanical loading has been demonstrated to be an important regulatory factor in alveolar bone homeostasis. It plays an essential role in maintaining the structure and mass of the alveolar processes throughout life (Pavlin and Gluhak-Heinrich, 2001).

On the other hand, skeletal unloading reduces proliferation and differentiation of osteoprogenitor cells *in vitro* (Kostenuik et al., 1997).

In vitro studies showed also that mechanical load increases the level of IGF-I in osteoblasts (McCarthy and Centrella, 2001). In addition, gene expression of IGF-I is detected during distraction osteogenesis and fracture healing (Bouletreau et al., 2002). In a recent study, it was demonstrated that IGF-I mRNA is twofold up-regulated in osteocytes of cortical bone after 6 h of mechanical loading, and thus supported the hypothesis that osteocytes are most important cells for the translation of mechanical stimuli into bone formation via IGF-I (Reijnders et al., 2007).

3. Aims and hypotheses

In this study should be investigated:

1. whether the locally produced IGF-I participate in the mechanotransduction process in the PDL during the early phase of tooth movement,
2. whether there is a differentiated expression pattern of IGF-I, IGF-IR and IRS1 in pressure and tensile sides,
3. and whether the distribution of IGF-I are dependent on the magnitude of the applied forces.

Moreover, the possible role of IGF-I in the remodeling of PDL during the early phase of orthodontic tooth movement should be also discussed.

4. Materials and methods

4.1 Animals

Twelve-week-old male Wistar rats each weighing 300–350 g (Harlan Winkelmann, Borchen, Germany) were used as experimental animals. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the local district government (Cologne) and the Animal Care Commissioner of the University of Bonn (Germany).

4.2 Experimental procedure

According to the experimental protocol of Kawarizadeh et al. (2004), rats were anaesthetized with 0.01 ml Rompun (Bayer, Leverkusen, Germany) and 0.24 ml Ketavet (Pharmacia and Upjohn, Erlangen, Germany). The animals were clamped into a head-holding device, and the occlusal surface of the maxillary right first molar was prepared by grinding a small hole with a dental diamond bur. The tooth surface was then treated with self-etching bonding material (Xeno III, Dentsply DeTrey, Konstanz, Germany) for 60 s. An orthodontic appliance consisting of a T-loop (0.016 _ 0.022-in. stainless steel wire, Ormco Corp., Glendora, CA, USA) was placed between the molar and a high-resolution 3D force/torque transducer (ATI, Industrial Automation, Garner, NC, USA), which had a resolution of 0.0125 N for force and 0.0625 Nmm for torque.
The T-loop was fixed to the occlusal surface of the molar with light-curing composite (Tetric, Vivadent, Schaan, Liechtenstein) (Fig. 3, 4, 5).

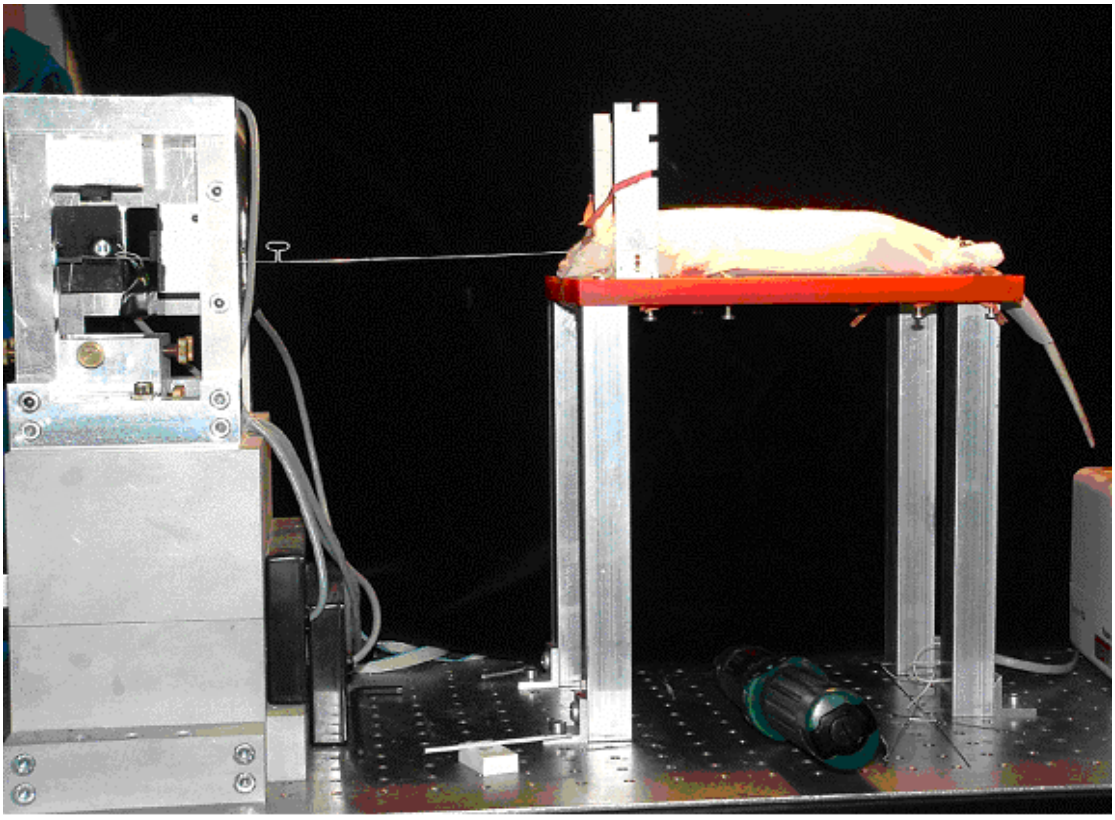

Figure 3: *Experimental procedure for the application of the orthodontic force system. The rats were clamped onto a head holding device, in order to place an orthodontic appliance consisting of a T-loop.*

Figure 4*: Placement of the appliance in the oral cavity of the rat.*

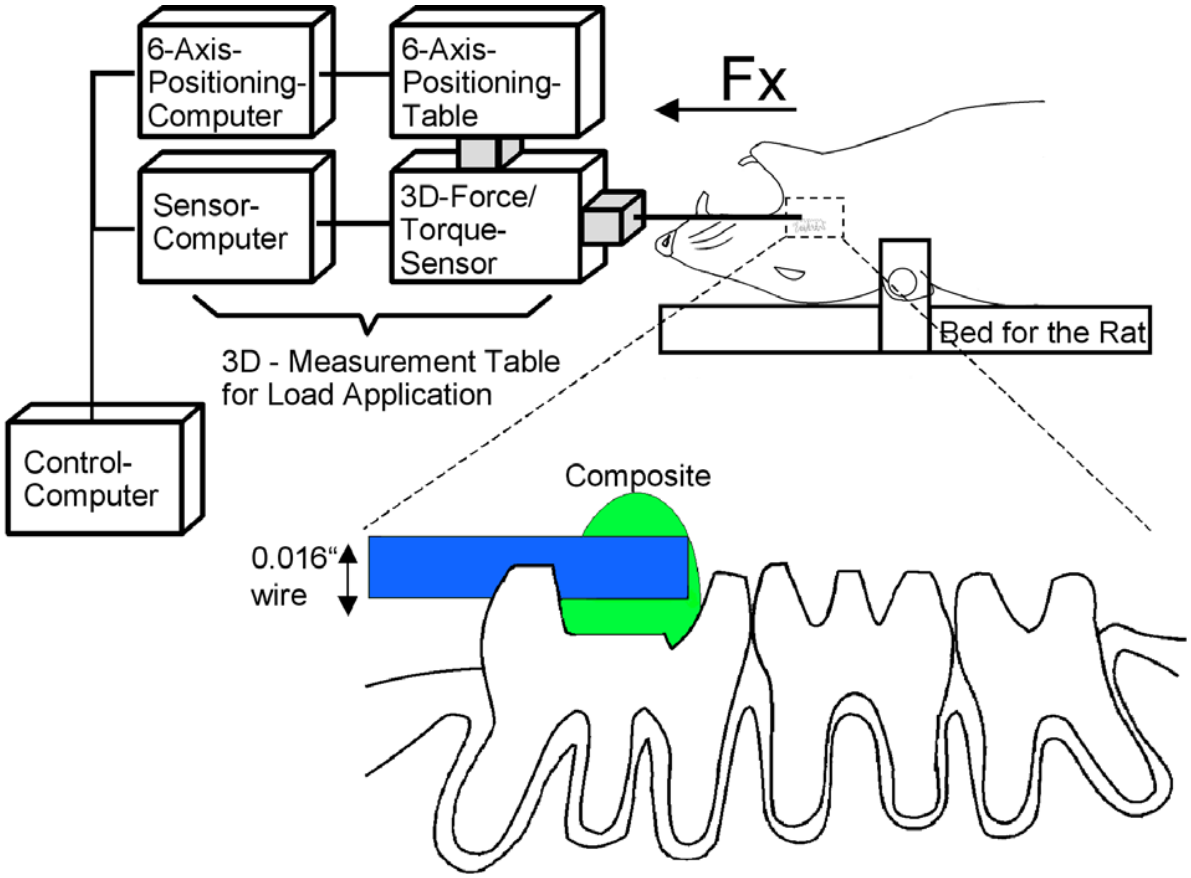

Figure 5*: Experimental set up: Diagrammatic representation of the experimental procedure for the application of the orthodontic force system. The force system was applied and measured by a 3D force/torque transducer, and mounted onto a 6-axis-positioning table. By moving the transducer, we applied loads to the first molar.*

In the first group of animals, constant forces for 4 hours of 0.1 N, 0.25 N and 0.5 N were applied in four animals each in order to move the molars mesially. The applied forces were kept constant and recorded continuously for 4 h (Fig. 6).

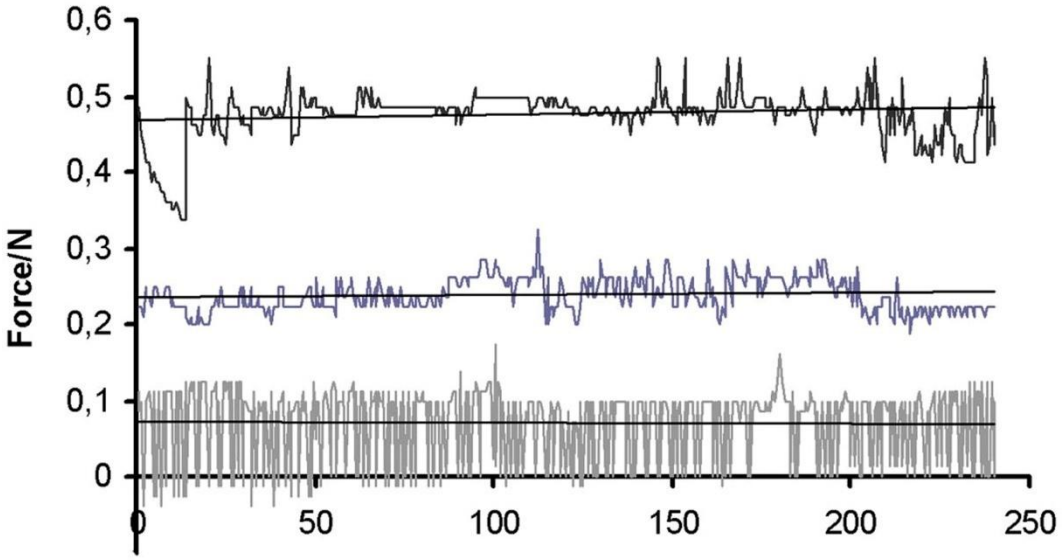

***Figure 6**: Presentation of a typical load-diagram: The force curves measured in the x-direction indicate a constant loading of the rat molar; these were approximately 0.1 N, 0.25 N and 0.5 N. The forces in the other directions were close to zero.*

In the second group, constant forces of 0.1 N were applied for 2 hours in 8 animals. Afterwards, the first and second molars were passively separated with composite and the occlusal contacts were eliminated by slicing the antagonistic lower molars. Four rats were killed after 1 day and four after 2 days. The untreated contralateral molars in 4 rats served as controls.

4.3 Histology

The anaesthetized animals were killed by an intravenous application of 2 ml T61 (embutramide mebezonium iodide; Intervet, Unterschleissheim, Germany) and decapitated. The maxillae were removed and dissected into right and left halves.
The soft tissues around the jawbone, except for the gingiva, were removed. The specimens were fixed in 4 % paraformaldehyde in 0.1 M phosphate buffer for 24 h and decalcified in 10 % ethylene diamine tetraacetic acid (EDTA) at room temperature for 5 weeks. After being dehydrated in ascending grades of ethanol, cleared in xylene, and paraffin-embedded, specimens were cut parasagittally with a microtome into 5-mm serial sections (HM 355s; Microm Int., Walldorf, Germany) and mounted on glass slides (K. Roth, Karlsruhe, Germany). Selected sections were stained with haematoxylin–eosin.

4.4 Immunohistochemistry

To establish representative regions of the periodontium and the adjacent alveolar bone, those sections were chosen that showed the medial root of the right upper first molar in maximal length. From these, three sections were taken at 30-mm intervals for quantitative analysis. Immunohistochemical staining was carried out with anti-IGF-I mouse monoclonal antibody (diluted 1:100, Biomol, Hamburg, Germany), polyclonal chicken anti- IGF-IR (diluted at 1:25, Biomol, Hamburg, Germany), and monoclonal mouse anti-IRS1 (diluted 1:300, Santa Cruz, Santa Cruz, USA). The sections were deparaffinized and rehydrated, rinsed with tris-hydroxymethyl aminomethane-buffered saline solution (TBS) at pH 7.4 for 10 min, and then soaked in methanol/H_2O_2 for 10 min in the dark, to block endogenous peroxidase activity.

In details the sections were treated with the following sequence:

1. xylol	10 min.
2. xylol	10 min.
3. 100 % ethanol	5 min.
4. 100 % ethanol	5 min
5. 90 % ethanol	5 min
6. 70 % ethanol	5 min
7. aqua dest	5 min
8. TBS	10 min
9. methanol/H2O2	20 min

Sections for IRS1 immunostaining were pretreated with goat serum (DAKO, Glostrup, Denmark) for 90 minutes. Antibodies were diluted in 1 % bovine serum albumin. The IGF-I antibody was applied in a humidity chamber for 1 hour at room temperature, the IGF-IR-and IRS1 antibodies overnight at 4 °C. Subsequently, sections were washed in TBS and incubated with suitable Envision+/HRP anti-mouse for IGF-I, anti-rabbit for IRS1 (Dako Cytomation, Hamburg, Germany) or anti-chicken for IGFIR (Rockland, Gilbersville, USA) diluted in 1 % TBS–BSA at 1:450, as secondary antibodies for 30 minutes in a humidity chamber at room temperature.

Antibody complexes were visualized using diaminobenzidine (DAB) for 10 minutes which yields a brown staining product. Thereafter, slides were rinsed, counterstained with Mayer's haematoxylin for 5 seconds, rinsed again, and mounted.

10. Goat serum only for IRS1-sections		90 min
11. Primary antibodies:		
	Dilution	
Monoclonal mouse anti-IGF-I	1:100	1Hour
Polyclonal chicken anti- IGF-IR	1:25	overnight
Monoclonal mouse anti-IRS1	1:300	overnight
12. TBS		10 min
13. Secondary antibodies:		
Envision+/HRP anti-mouse		30 min
Anti-goat immune globulin/HRP		30 min
Anti-chicken for IGFIR	1:450	30 min
14. TBS		10 min
15. Diaminobenzidine (DAB)		10 min
16. TBS		10 min
17. Mayer's hematoxylin		1 sec
18. 100 % ethanol		2 min
19. 100 % ethanol		2 min
20. xylol		2 min
21. xylol		2 min

Negative controls were prepared by omission of the primary antibodies from the staining procedures and replacing it with an isotype IgG using the identical concentration.

4.5 Histomorphometry and statistical analysis

Sections were scanned by means of a scanner camera (Axio Cam MRC; Zeiss, Göttingen, Germany) mounted on a light microscope (Axiophot 2; Zeiss, Göttingen, Germany), and viewed with imaging software (Axiovision; Zeiss, Göttingen, Germany) on a personal computer. Counting of the percentage of immuno-histochemically positive cells was performed in two separate predefined areas in every selected section. These areas were located mesio-coronally and disto-coronally to the medial root. Counts were made at a magnification of 400.

Means and standard deviations were calculated for each group of four rats. Hundred sections were randomly selected from the original samples and remeasured by the same operator after a time interval of 3 months without reference to the previous measurements.

The casual error was calculated according to Dahlberg's formula:

$$Sx = \sqrt{\frac{\sum D^2}{2N}}$$

Where Sx is the error of the measurement, D is the difference between duplicated measurements, and N is the number of double measurements.

The percentage of IGF-I-, IGF-IR- and IRS1-positive cells was calculated with Excel software. The statistical analyses were performed with SPSS Software, version 17 (SPSS Inc. Chicago, IL). The normality test was used to estimate the normal distribution of the data. The percentage of positive cells for all tested factors was compared using post hoc test (Bonferroni). The level of statistical significance was set at $p < 0.05$.

4.6 The used chemicals, reactors and instruments

4.6.1 List of the chemicals:

Acetone	Otto Fischer, Saarbrücken, Germany
Ethanol (70-100 %)	Merck, Münster, Germany
Embedding medium DePex	Serra, Heidelberg, Germany
3,3-diaminobenzidin(DAB)	Sigma, Steinheim, Germany
Ethylene diamine tetraacetic acid (EDTA)	Calbiochem, Darmstadt, germany
Eosin solution 1 %	Merck, Darmstadt, Germany
Formic acid	Merck, Darmstadt, Germany
Formaldehyde 40 %	Merck, Darmstadt, Germany
Hematoxylin	Merck, Darmstadt, Germany
Methanol	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodiumsulfate solution	Merck, Darmstadt, Germany
Paraffin-Histo-comp	Vogel, Giessen, Germany
Hydrochloride 2 mol/l	Merck, Darmstadt, Germany
Serumalbumin from bovine	Paesel and Lor, Frankfurt, Germany
Tris	ICN Biomedicals, Ohio, USA
Xylol original	Merck, Darmstadt, Germany
Xylol substitute XEM-200	Vogel, Giessen, Germany

4.6.2 List of the reactors:

TBS-solution:

-9.0 gr NaCl

-6.0 gr Tris

-1000 ml aqua dest. + 2 ml HCl pH 7.6

TBS-BSA-solution:

-0.1 gr serumalbumin from bovine

-10 ml TBS-solution

EDTA:

-200 gr EDTA

-68 gr Tris

-2000 ml aqua dest

4.6.3 List of the instruments:

Analysis scale	Sartorius, Göttingen, Germany
Thermo cupboard	Memmert, Schwabach, Germany
Cover glass	Engelbrecht, Edermunde, Germany
DigitalpH-meter 197	WTW, Vienna, Austria
Eppendorf pipettes	Eppendorf, Hamburg, Germany
Light microscope Axioskop 2	Zeiss, Göttingen, Germany
Axio Cam MRC	Zeiss, Göttingen, Germany
Rotation microtome HM 3559	Microtom, Walldorf, Germany
Superfrost plus slides	Menzel, Braunschweig, Germany
Warm plate	Medax Nagel, Kiel, Germany

5. Results

5.1 Histology

Orthodontic loading resulted in stretching of the periodontal fibres on the disto-coronal aspects of the medial root and compression of the ligament on the mesio-coronal aspects of the medial root (Fig. 7).

In the first group, where constant forces for 4 hours of 0.1 N, 0.25 N and 0.5 N were applied in four animals each, tension and compression sides looked almost the same because of the shortness of the experiment.

On the tension side, especially those teeth to which a high force (0.5 N) had been applied showed obvious structural disturbance of PDL fibres and some sections howed pyknosis of PDL cells (Fig. 17B). In the teeth for which lower forces had been used, this phenomenon was less obvious. In the second group, in which the rats were killed after 1day and 2 days from application of constant forces for 2 hours, the mesial side of PDL was compressed and the distal PDL widened.

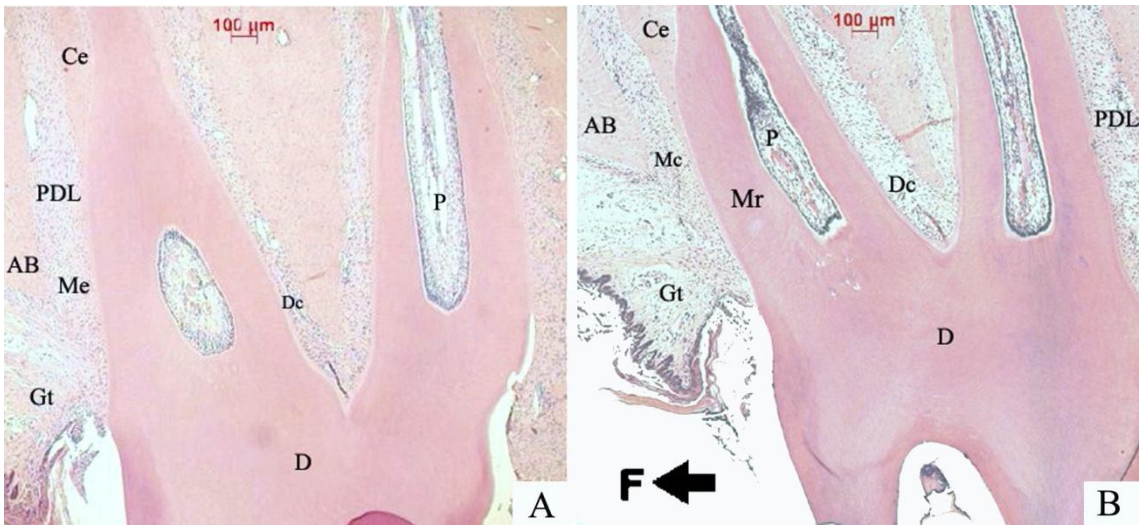

Figure 7: *(A, B) Overview: Experimentally loaded right first molar (B) and untreated contralateral molar (A). Arrow indicates the force direction. HE staining; magnification 50x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; D, dentin; P, pulp; Mr, medial root; Gt, gingival tissue; Mc, mesial coronal; Dc, distal coronal.*

Figures 15A and 19A show blood vessels in various sizes in the sections of PDL.

Osteoclasts were seen on the alveolar bone surface in the disto-coronal areas of untreated rats (Fig. 10A, 19A), after 24 and 48 hrs from force application, no more osteoclasts were found.

The total number of PDL cells in the tension side increased significantly compared with the control group especially after 24 hrs and decreased significantly in the pressure side (Fig. 8).

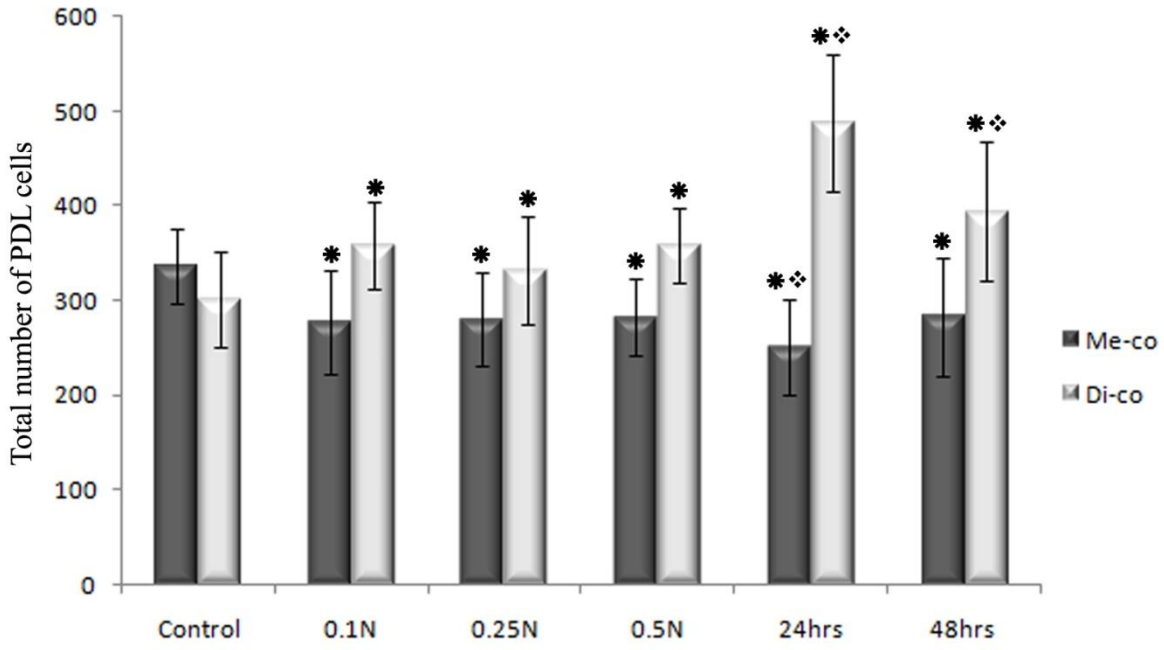

***Figure 8**: Total numbers of the PDL-cells, expressed as pillars, dark-coloured pillars represent mesio-coronal areas and light-coloured ones represent disto-coronal areas. The results are expressed as means and standard deviations. Symbols indicate the significant difference ($p < 0.05$) between experimental groups and the control group (✱) or among marked groups and the other groups (❖).*

5.2 Immunohistochemistry and Histomorphometry

In the control group, immunoreactivity for all investigated proteins was distributed throughout the examined PDL-areas, but tended to be localized more on the mesial side (12A, 17A, 21A) than on the distal side of the medial root (10A, 15A, 19A). Immunoreactivity was observable in osteoblasts, fibroblasts, osteoclasts and cementoblasts. According to Dahlberg's formula, the casual error was 3.65. The unwanted background staining, which is one of the most common problems in immunohistochemistry, was reduced by blocking the endogenous peroxidase activity for 10 min with H_2O_2. As expected, no immunostaining appeared under the negative control conditions (Fig. 9).

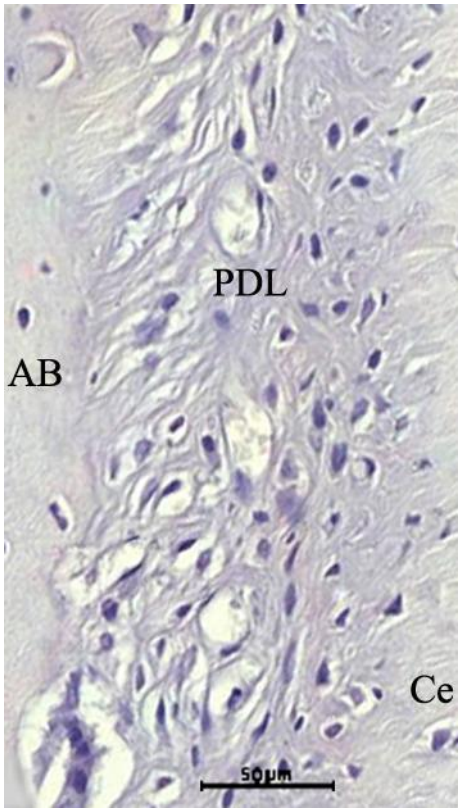

Figure 9: negative control for IGF-I. Magnification 400x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum.

5.2.1 Results for IGF-I

As a reaction to load, changes in immunoreactivity were seen for all proteins examined on both the mesial and distal sides of the PDL. The number of IGF-I positive PDL cells on the tension side (disto-coronal) was found to be significantly increased in the both groups in comparison with the controls (Fig. 10, 11, 14). The differences between

the groups, where constant forces for 4 hrs of 0.25 N and 0.5 N were applied, and the group, where the rats were killed after 48 hrs, were significantly recognized. In the pressure side (mesio-coronal), the number of IGF-I-positive cells was significantly decreased in the 4 hrs group (Fig. 12, 13A, 14). This decrease tended to be dependent on the force magnitude. After 24 and 48 hrs the number of the immunopositive cells increased to the mean level of the control group in the mesio-coronal areas. Thus, the differences between the first and second group were significant with one exception. No significant difference was seen between the group, where 0.1N were applied for 4 hrs, and the 24 hrs group (Fig. 13B).

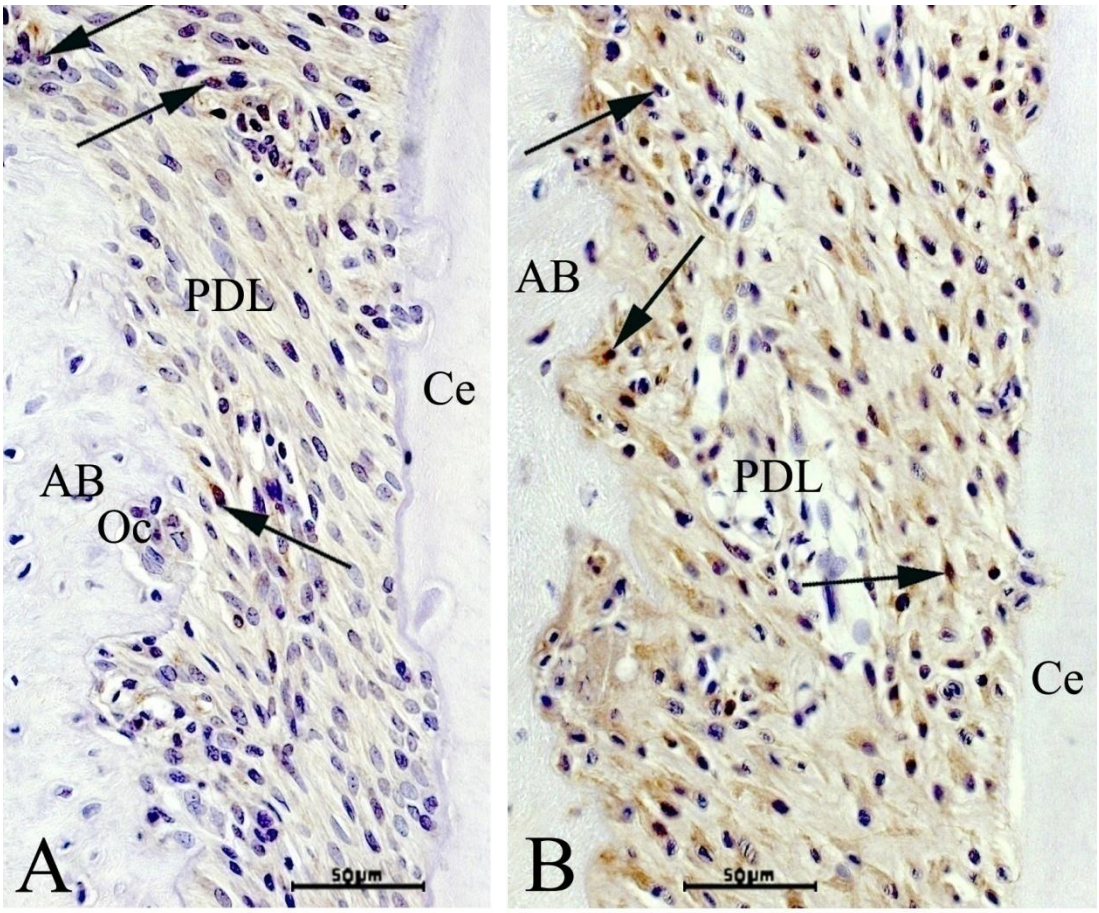

Figure 10: *(A, B) Representative views of immunolabelling for IGF-I on the disto-coronal aspects in the PDL of the medial root. The immunolabelling of IGF-I represented in control tooth (A) and in tooth treated with force of 0.1N for 4hrs (B). The immunostained cells appear brown (arrows). The labelled cells are regularly distributed in the periodontal ligament. DAB staining; magnification 400 x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; Oc, osteoclast; arrows, immunopositive cells.*

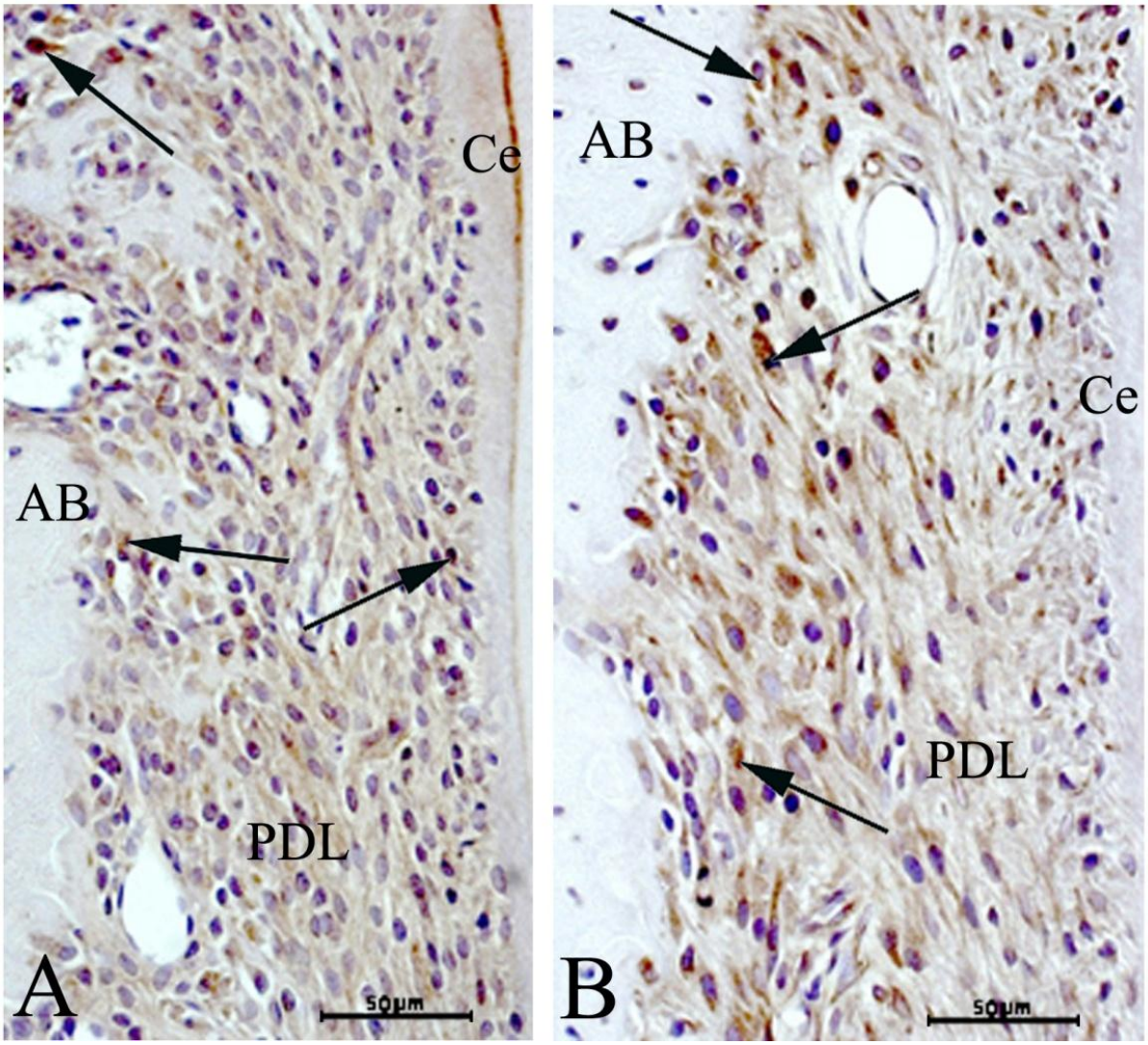

Figure 11: *(A, B) Representative views of immunolabelling for IGF-I on the disto-coronal (tension side) aspects in the PDL of the medial root. The immunolabelling of IGF-I was represented in teeth treated with the mentioned loading regimes for 24 hrs (A) and 48 hrs (B). The immunostained cells appear brown (arrows). The labelled cells are regularly distributed in the periodontal ligament. DAB staining; magnification 400 x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; arrows, immunopositive cells.*

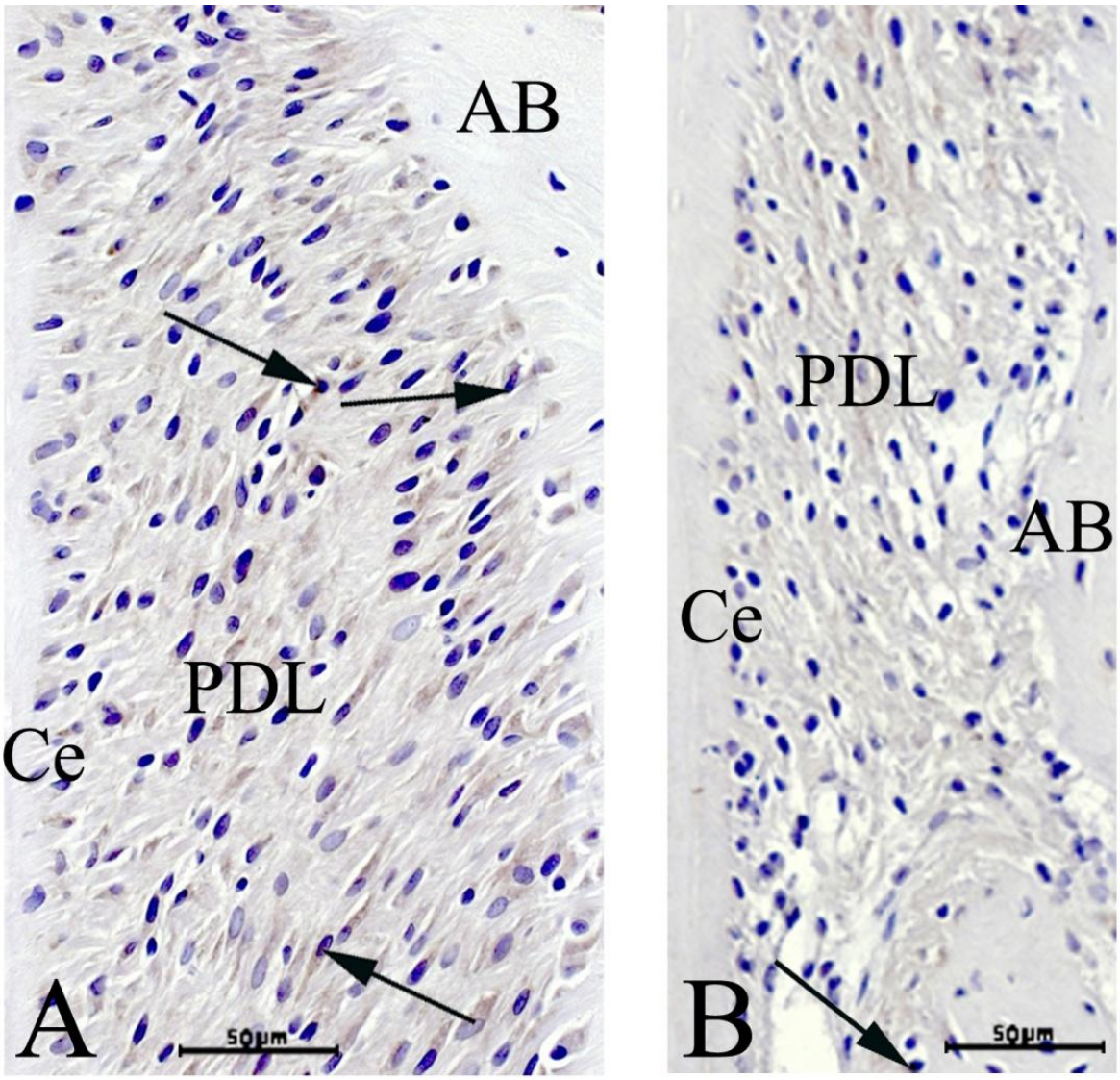

Figure 12: *(A, B) Representative views of immunolabelling for IGF-I on the mesio-coronal aspects in the PDL of the medial root. The immunolabelling of IGF-I was represented in control tooth (A) and in tooth treated with force of 0.1N for 4hrs (B). The immunostained cells appear brown (arrows). The labelled cells are regularly distributed in the periodontal ligament of the control tooth and significantly reduced in the periodontal ligament of the treated tooth. DAB staining; magnification 400 x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; arrows, immunopositive cells.*

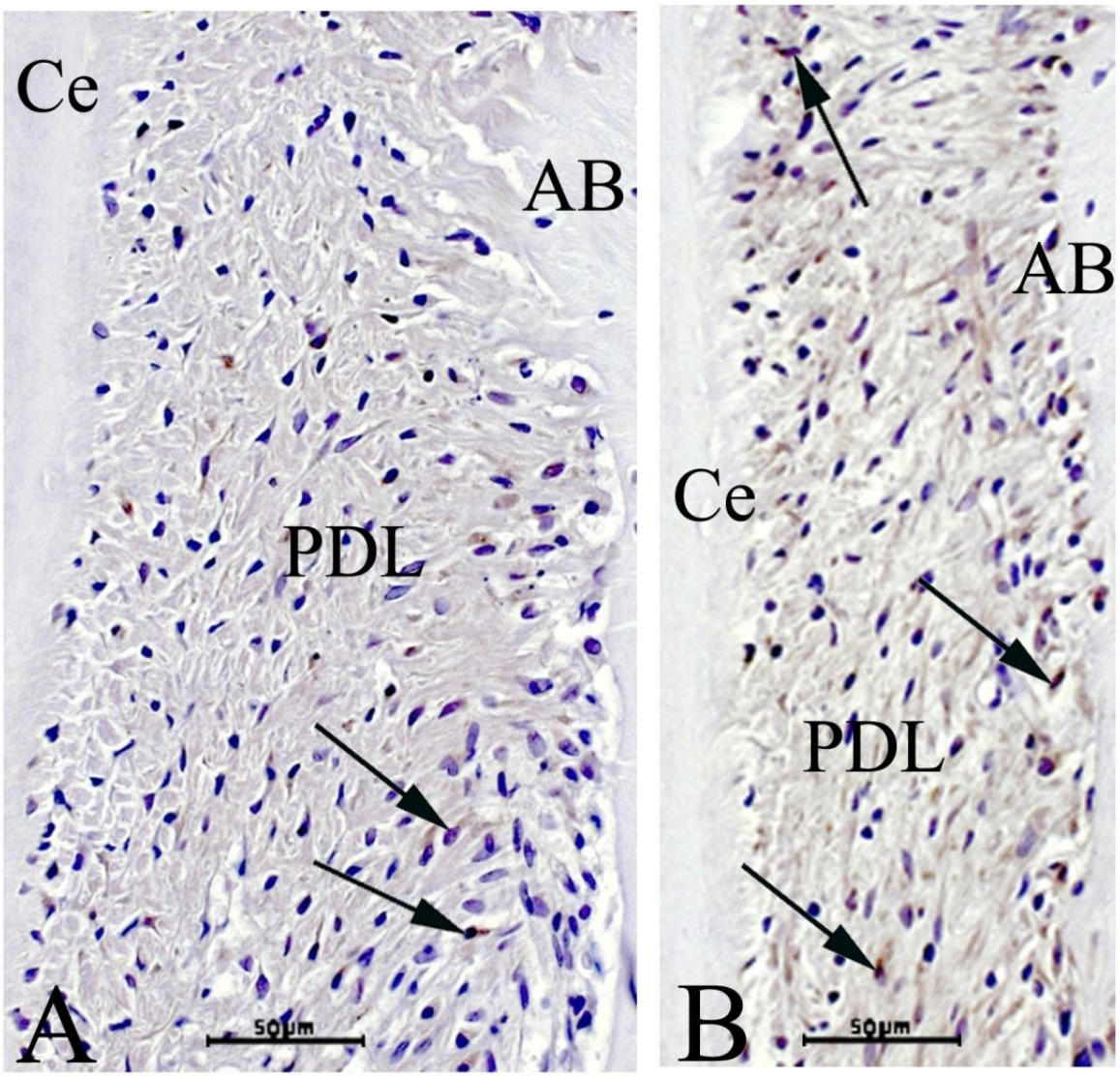

Figure 13: *(A, B) Representative views for immunolabelling of IGF-I on the mesio-coronal (pressure side) aspects in the PDL of the medial root. The immunolabelling of IGF-I was represented in teeth treated with forces of 0.1N for 4hrs (A) and with the mentioned loading regimes for 48 hrs (B). The immunostained cells appear brown (arrows). The labelled cells are regularly distributed in the periodontal ligament. DAB staining; magnification 400 x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; arrows, immunopositive cells.*

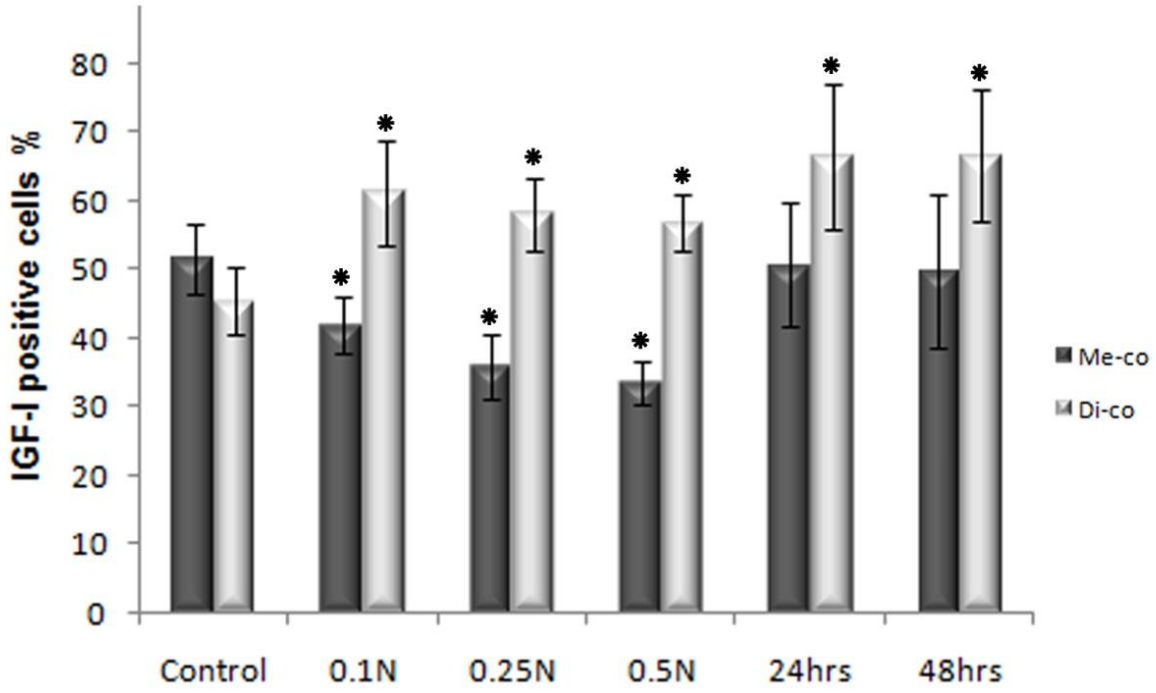

Figure 14: *Percentage of the numbers of the IGF-I-positive cell. They are expressed as pillars, the dark-coloured pillars represent the mesio-coronal areas and the light-coloured ones represent the disto-coronal areas. Each pillar represents a group of four animals treated with the mentioned loading regimes. The immunopositive cells were counted as percentages of the total number of cells. Results are representative of 3 sections of each animal. For each of the 4 specimens, a total of 2 fields in 3 sections were analyzed. The results are expressed as means and standard deviations. The symbol (✱) indicates a significant difference ($p < 0.05$) between experimental groups and the control group.*

5.2.2 Results for IGF-IR

As seen by IGF-I, the number of immunopositive cells of IGF-IR on the tension side (disto-coronal) was found to be significantly increased in the both groups in comparison with the controls (Fig. 15, 16, 18). This increase of the immunopositive cells in 4 hrs groups was negative dependent on the force magnitude. The difference between groups, where forces of 0.1 N and 0.5 N for 4 hrs were applied, was significant. After 24 hrs, the IGF-IR-positive cells reached their peak point and tend to decrease slightly after 48hrs. Significant differences between the groups, where constant forces for 4 hours of 0.25 N and 0.5 N were applied, and the 48 hrs group were observed.

At the pressure side (mesio-coronal), the significant effect of the force application on the number of IGF-IR-positive cells was not dependent on experimental time but on the force magnitude. The number of IGF-IR-positive cells was significantly decreased in groups treated with forces of 0.25N and 0.5N, whereas the group treated with light force of 0.1N showed no significant differences compared to control group in the mesio-coronal areas (Fig. 17, 18). Therefore, the differences between the groups, where 0.5N for 4 hrs was applied, and the second group (24 hrs and 48 hrs) were also significant.

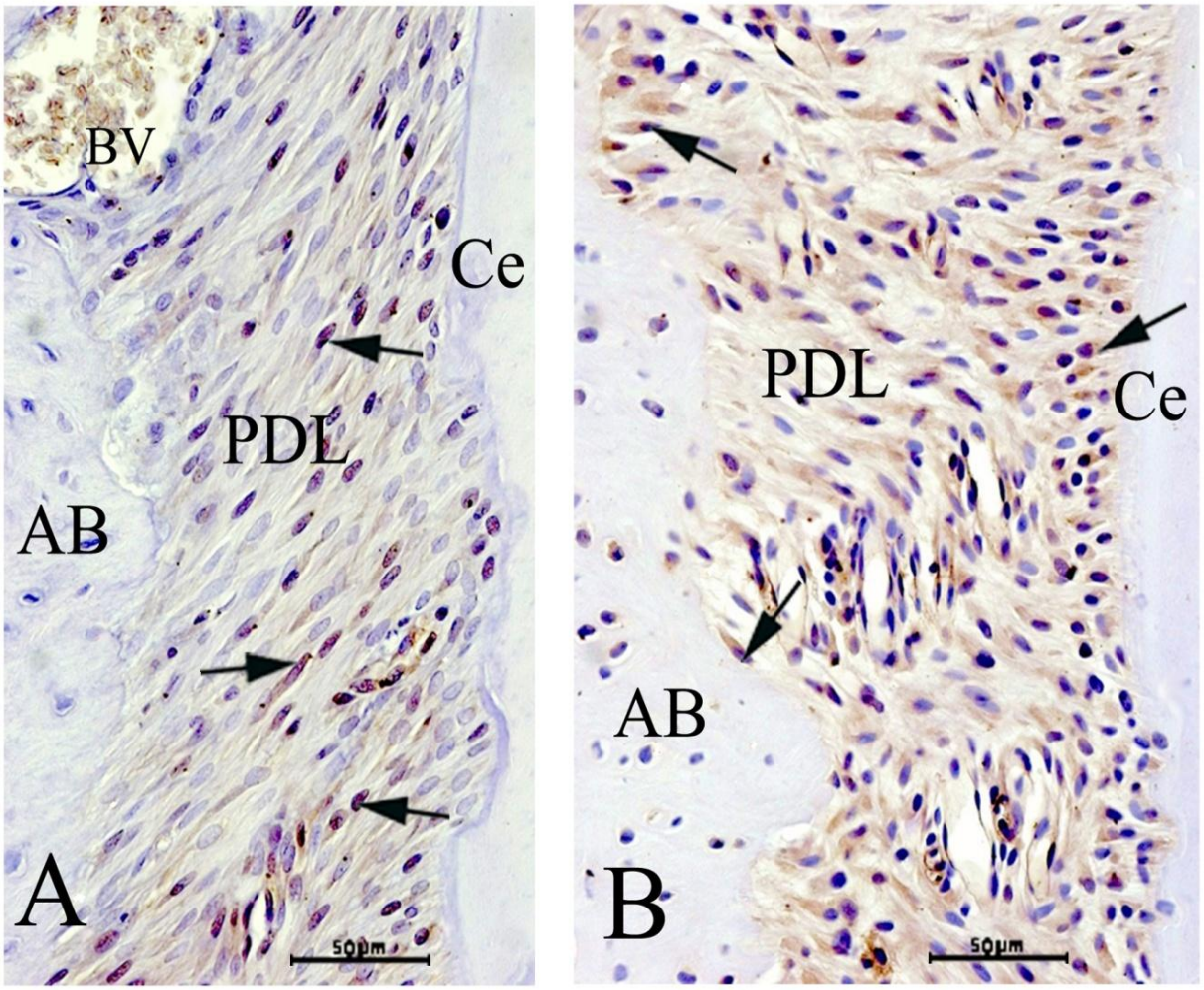

***Figure 15**: (A, B) Representative views for immunolabelling of IGF-IR on the disto-coronal aspects in the PDL of the medial root. The immunolabelling of IGF-I was represented in control tooth (A) and in tooth treated with force of 0.1N for 4hrs (B). The immunostained cells appear brown (arrows). The labelled cells are regularly distributed in the periodontal ligament. DAB staining; magnification 400 x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; BV, blood vessel; arrows, immunopositive cells.*

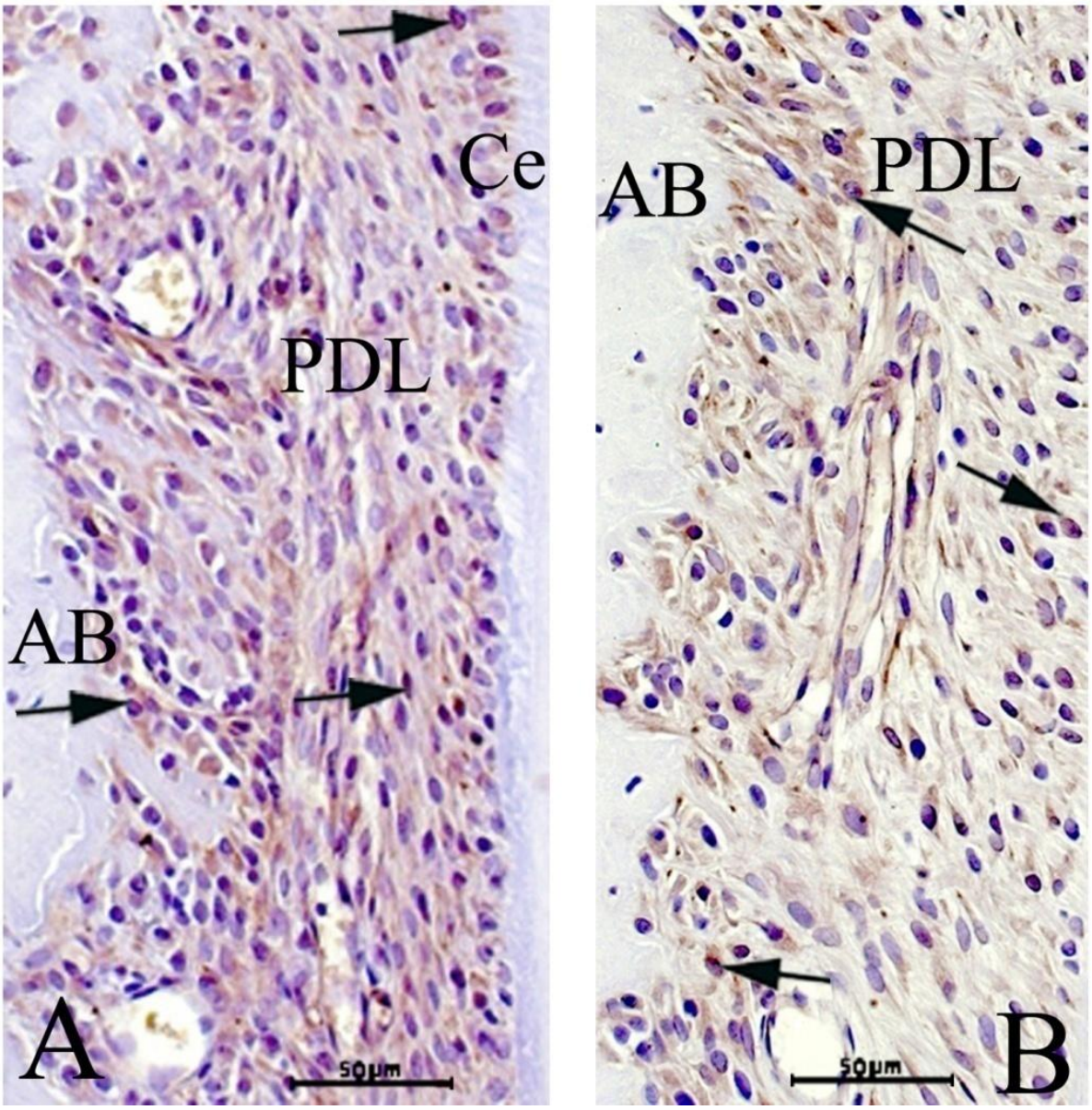

Figure 16: *(A, B) Representative views for immunolabelling of IGF-IR on the disto-coronal (tension side) aspects in the PDL of the medial root. The immunolabelling of IGF-I was represented in teeth treated with the mentioned loading regimes for 24 hrs (A) and 48 hrs (B). The immunostained cells appear brown (arrows). The labelled cells are regularly distributed in the periodontal ligament. DAB staining; magnification 400 x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; arrows, immunopositive cells.*

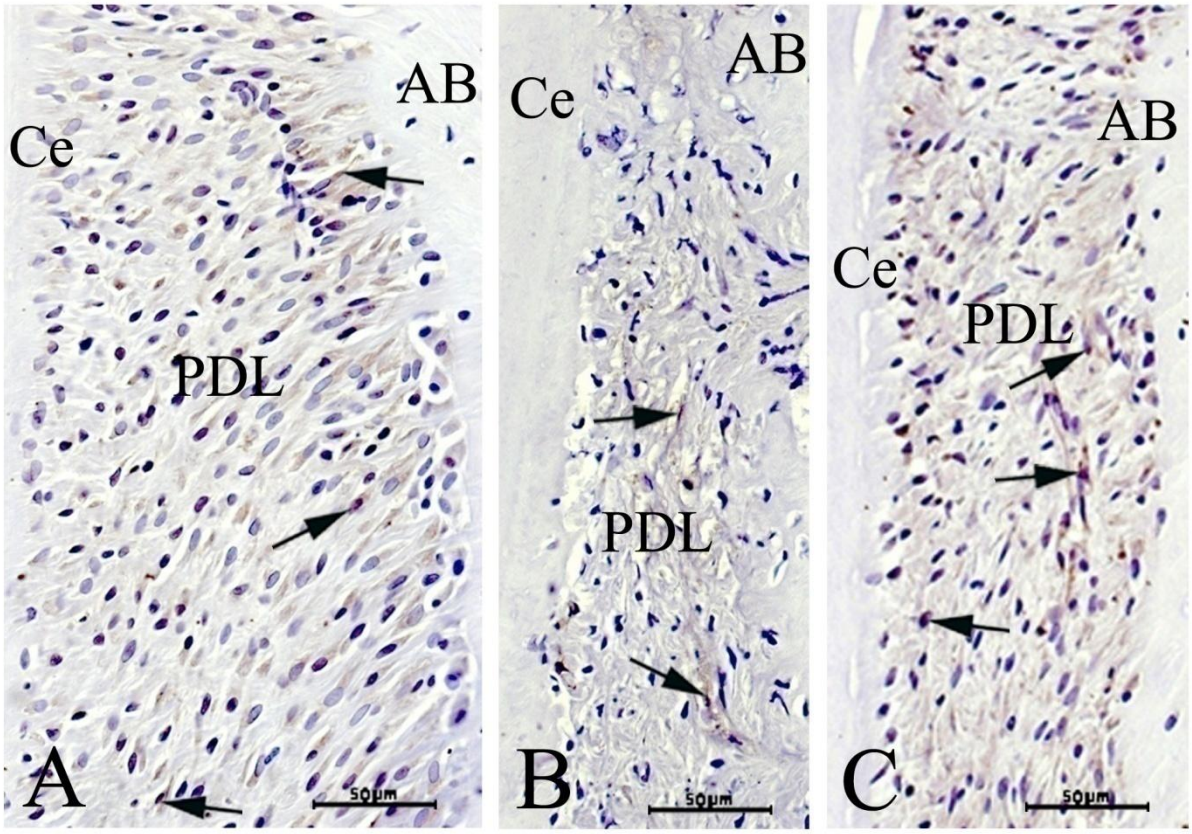

Figure 17: *(A, B, C) Representative views for immunolabelling of IGF-IR on the mesio-coronal (pressure side) aspects in the PDL of the medial root. The immunolabelling of IGF-IR was represented in control tooth (A), in teeth treated with forces of 0.5N for 4hrs (B) and with the mentioned loading regimes for 48 hrs (B). The immunostained cells appear brown (arrows). The labelled cells are regularly distributed in the periodontal ligament. DAB staining; magnification 400 x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; arrows, immunopositive cells.*

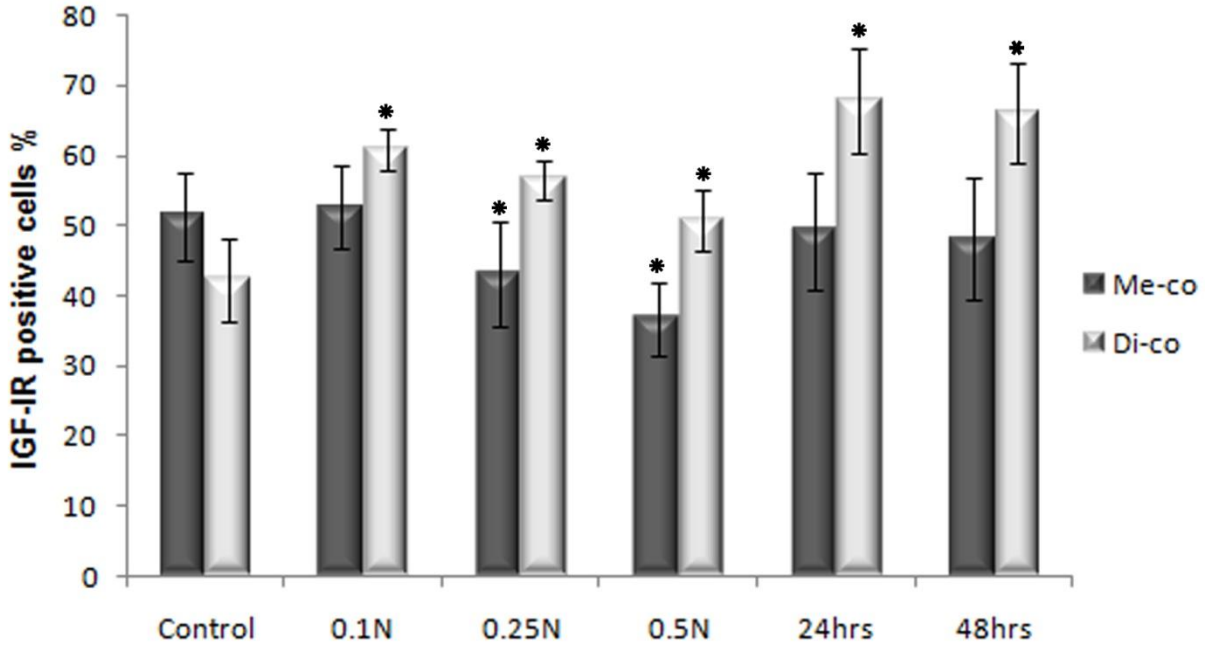

Figure 18: *Percentage of numbers of IGF-IR-positive cells. They are expressed as pillars, the dark-coloured pillars represent the mesio-coronal areas and the light-coloured ones represent the disto-coronal areas. Each pillar represents a group of four animals treated with the mentioned loading regimes. The immunopositive cells were counted as percentages of the total number of cells. Results are representative of 3 sections of each animal. For each of the 4 specimens, a total of 2 fields in 3 sections were analyzed. The results are expressed as means and standard deviations. The symbol (✱) indicates a significant difference ($p < 0.05$) between experimental groups and the control group.*

5.2.3 Results for IRS1

As seen by IGF-I and IGF-IR, the number of immunopositive cells of IRS on the tension side (disto-coronal) was found to be significantly increased in the both groups in comparison with the controls (Fig. 19, 20, 22).
In the 24 hrs group, the IRS1-positive cells reached their peak point and decreased then significantly in the 48 hrs group. Significant differences between the first group, where constant forces for 4 hours of 0.1N, 0.25 N and 0.5N were applied, and the 24 hrs group, were found. Additionally, the difference between the group, where 0.5N force for 4 hrs was applied, and 48 hrs group, was also significant.

In the pressure side (mesio-coronal), the number of IRS1-positive cells decreased significantly in the 4 hrs group. Thereafter, the number of the positive cells increased in the second group to the mean level of the control group in the mesio-coronal areas. Therefore, the differences between the first group (4hrs) and second Group (24 and 48 hrs) were significant (Fig. 21, 22).

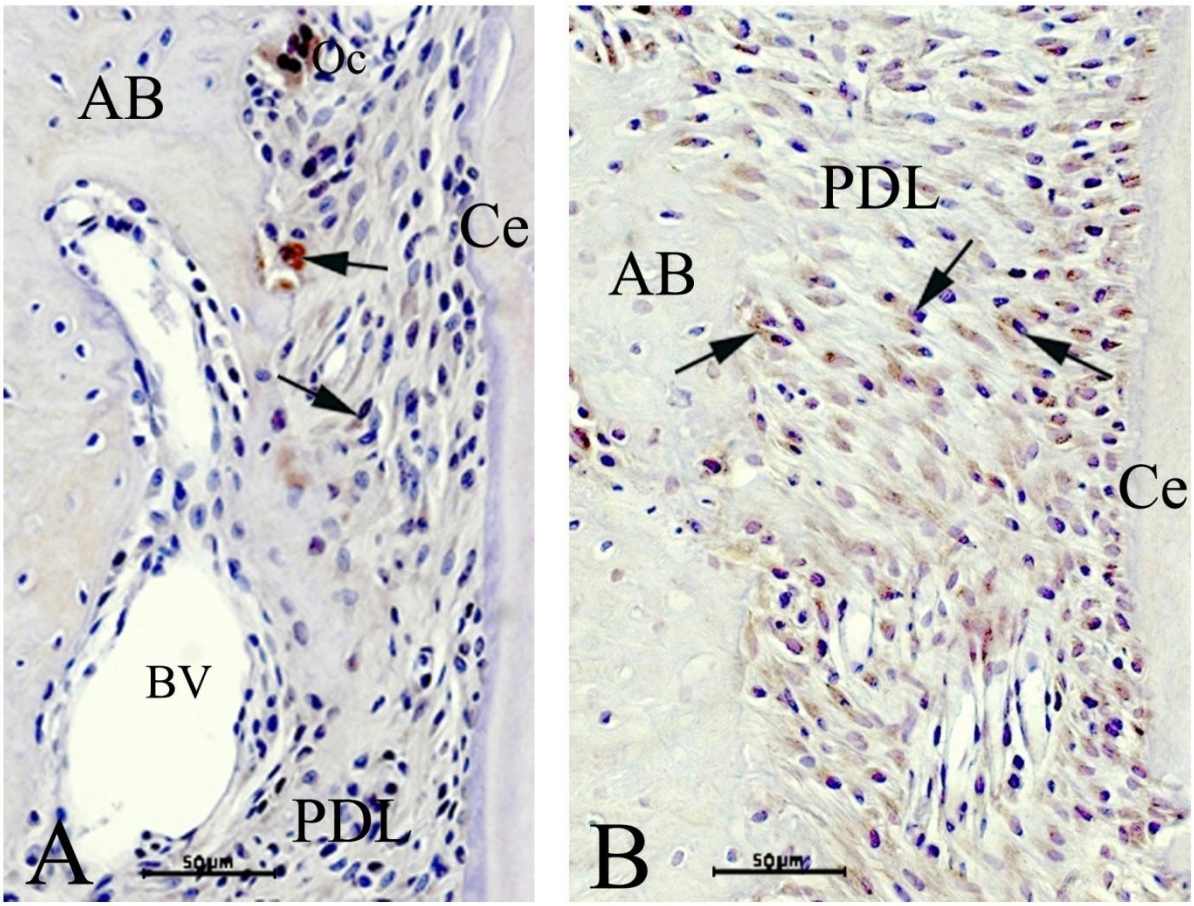

***Figure 19**: (A, B) Representative views for immunolabelling of IRS1 on the disto-coronal aspects in the PDL of the medial root. The immunolabelling of IRS1 was represented in control tooth (A) and in tooth treated with force of 0.1N for 4hrs (B). The immunostained cells appear brown (arrows). The labelled cells are regularly distributed in the periodontal ligament. DAB staining; magnification 400 x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; BV, blood vessel; Oc, osteoclast; arrows, immunopositive cells.*

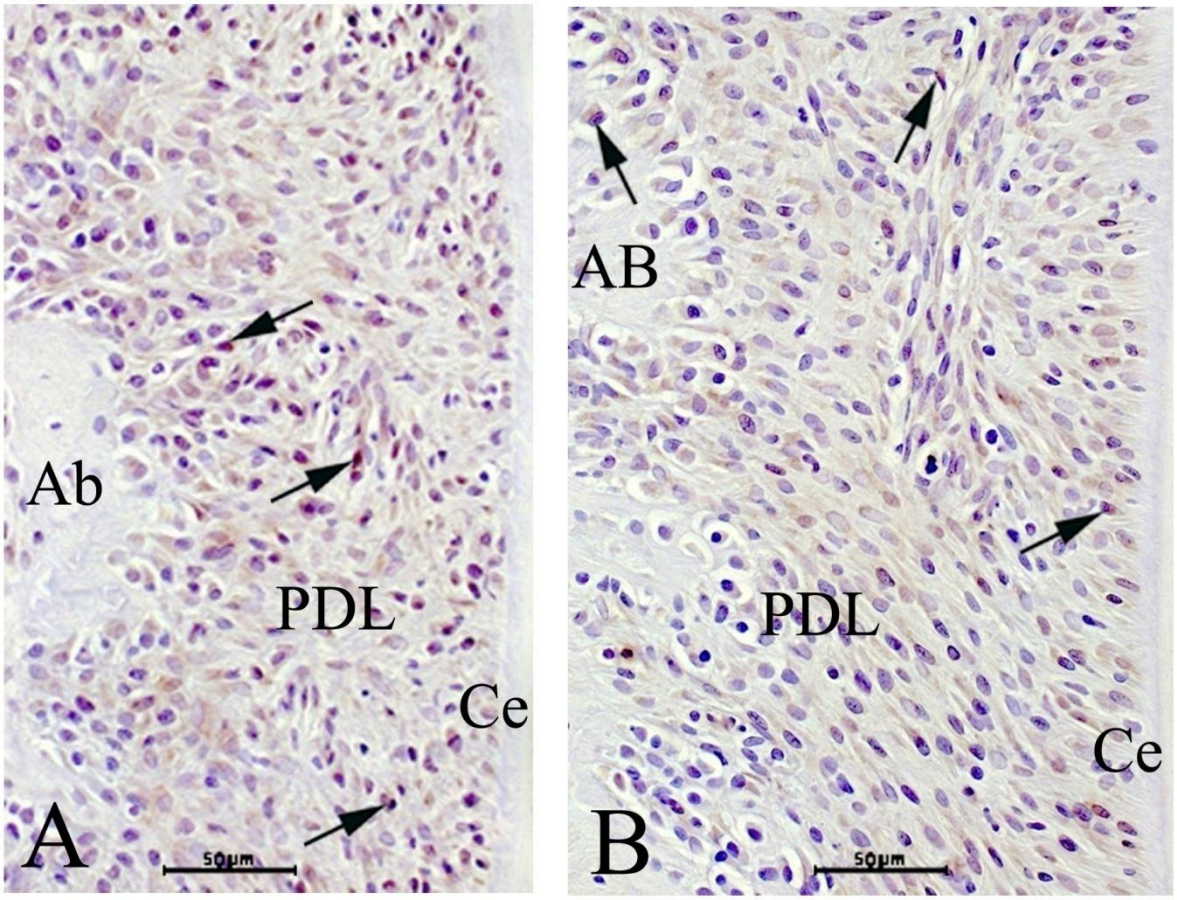

Figure 20: *(A, B) Representative views for immunolabelling of IRS1 on the disto-coronal (tension side) aspects in the PDL of the medial root. The immunolabelling of IRS1 was represented in teeth treated with the mentioned loading regimes for 24 hrs (A) and 48 hrs (B). The immunostained cells appear brown (arrows). The labelled cells are regularly distributed in the periodontal ligament. DAB staining; magnification 400 x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; arrows, immunopositive cells.*

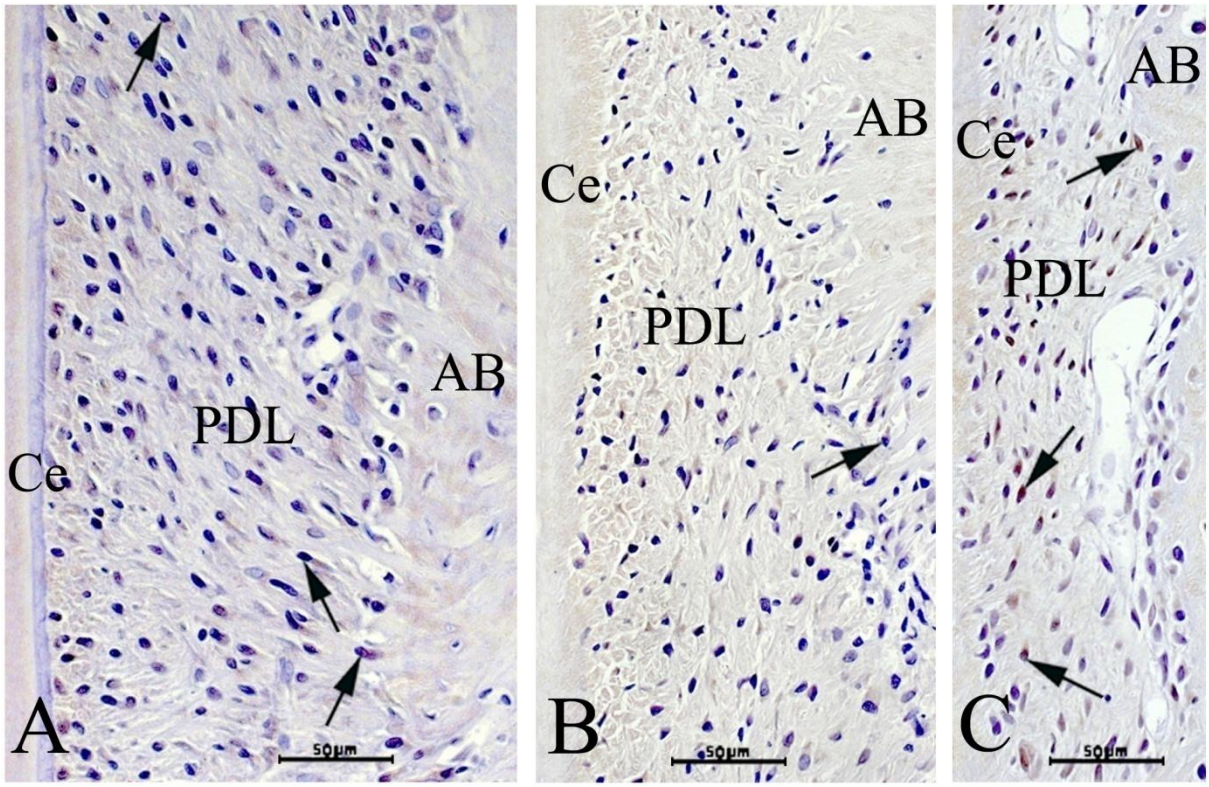

Figure 21: *(A, B, C) Representative views for immunolabelling of IRS1 on the mesio-coronal (pressure side) aspects in the PDL of the medial root. The immunolabelling of IRS1 was represented in control tooth (A), in teeth treated with forces of 0.5N for 4hrs (B) and with the mentioned loading regimes for 48 hrs (B). The immunostained cells appear brown (arrows). The labelled cells are regularly distributed in the periodontal ligament. DAB staining; magnification 400 x; AB, alveolar bone; PDL, periodontal ligament; Ce, cementum; arrows, immunopositive cells.*

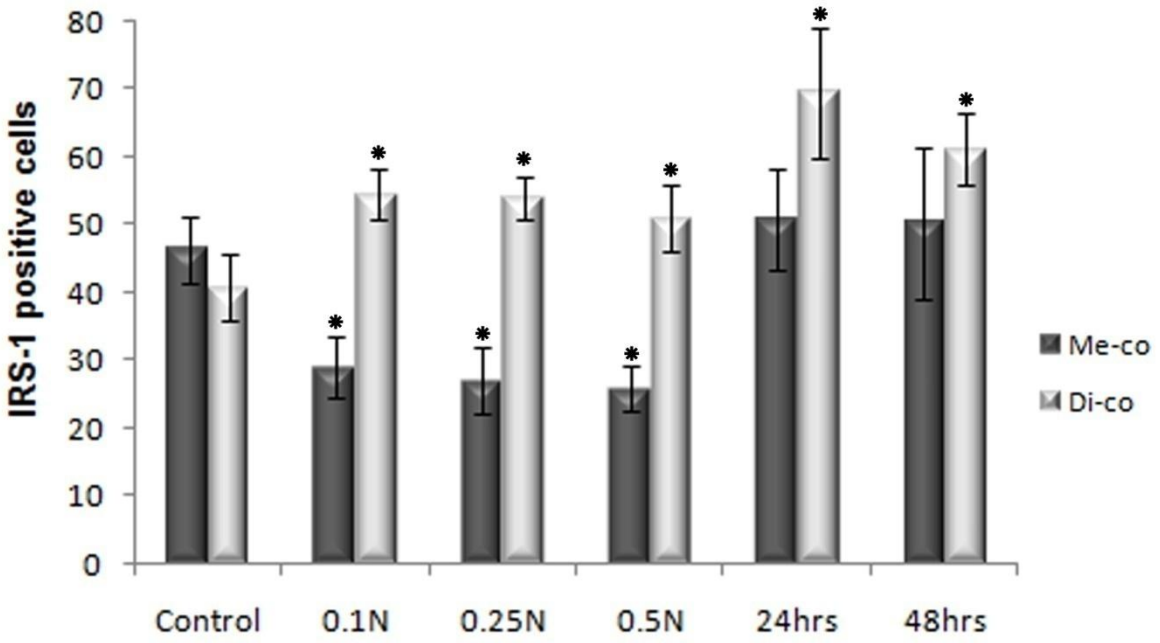

Figure 22: *Percentage of numbers of IRS1-positive cells. They are expressed as pillars, the dark-coloured pillars represent the mesio-coronal areas and the light-coloured ones represent the disto-coronal areas. Each pillar represents a group of four animals treated with the mentioned loading regimes. The immunopositive cells were counted as percentages of the total number of cells. Results are representative of 3 sections of each animal. For each of the 4 specimens, a total of 2 fields in 3 sections were analyzed. The results are expressed as means and standard deviations. The symbol (✱) indicates a significant difference ($p < 0.05$) between experimental groups and the control group.*

6. Discussion

The present study was performed to investigate the occurrence and distribution of insulin-like growth factor-I (IGF-I), IGF-IR and IRS1 in the periodontal ligament (PDL) during the early stage of orthodontic tooth movement. The study was carried out using forces of varying amount and different time periods in an animal model.

This study showed that IGF-I, IGF-IR and IRS1were distributed locally in the control group throughout the PDL and tended to be localized more on the mesial side than the distal side of the medial root.

In the PDL of human deciduous and permanent teeth as well as in the PDL of rats, Götz et al. have studied the occurrence of the IGFs (Götz et al., 2001; 2006a; 2006 b). They found that the PDL, in particular, contains mostly all IGF components.

They have suggested that IGFs might be stored in the PDL matrix via their binding proteins linked to glycosaminoglycans and regulate through autocrine and paracrine pathways the PDL cell survival, proliferation and matrix turnover.

Although the liver is the main source of IGF-I and the absence in its gene causes growth retardation in mice and humans, many cell types produce and respond to IGF-I via autocrine or paracrine actions (Woods et al., 1996, Bonapace et al., 2003; Barton and Crowder, 2010; Netchine et al, 2011). For example, IGF-I is one of the best characterized and the most abundant growth factors in the bone tissue (Mohan and baylink, 1991; Canalis, 2009). This local production of IGF-I can even compensate the lack of the liver IGF-I in genetically manipulated mice (andrew et al., 2001), suggesting that autocrine/paracrine-produced IGF-I is the main determinate of postnatal body growth (Ohlsson et al., 2000).

The previous investigations and the results of this study strength the crucial role of autocrine and paracrine IGF-I and its impacts in the biology of PDL.

This study elucidates that the application of precise short term loading at teeth in rats leads to an increase in IGF-I, IGF-IR and IRS1 expression at the tension side.

This observation is in accordance with other studies which showed that loading at different tissues increases the production of IGF-I: Human movement occurs from the

force created by contracting muscles, which is transmitted to bone via tendon, all these three tissues can respond to the mechanical loading by producing local IGF-I or its isoform.

In skeletal muscles, insulin-like growth factor I (IGF-I) play a critical role in their formation, maintenance, and regeneration. Longitudinal exercise studies have shown that it is possible to increase muscle strength and performance without concomitant and robust changes in circulating IGF-I. Such studies indicating that the effect of exercise on skeletal muscles is mediated via paracrine/autocrine IGF-I rather than endocrine IGF-I (Frystyk, 2010). Goldspink (Goldspink, 1999) showed that loading increases the production of IGF-I in muscle cells, functioning in an autocrine/paracrine mode, is an important mediator of skeletal muscle adaptation being sensitive to increases in loading (Adams, 2002).

In human tendon, which resembles the PDL in its function, it can be demonstrated that an increase in the interstitial concentration of IGF-I and its binding proteins takes place after exercise. The increase in IGF-I expression in tendon includes the isoform that has so far been thought only to exist in skeletal muscle (mechano-growth factor) (Kjaer et al., 2009). In rats, it was suggested that the IGF-I and its splice variant mechano-growth factor (MGF) could be involved in collagen synthesis in tendon in response to mechanical loading (Olesen et al., 2006).

The transmitted mechanical stimulation to bone leads to increased bone formation and plays an essential role in maintaining skeletal integrity. Growth factors and osteocytes, which act as mechanosensors, play a key role during the bone formation after mechanical stimulation. Recently, Reijnders et al. (2007) have found that IGF-I mRNA is upregulated within endocortical osteocytes of the shaft of rat tibia 6 h after mechanical loading. They suggested that IGF-I, which is located in osteocytes, is involved in the translation of mechanical stimuli into bone formation. It has been also demonstrated that rat osteoblasts respond to the mechanical forces which may regulate their activities indirectly by promoting the autocrine effect of IGF-1 (Xian et al., 2007).

The ability of IGF-I to translate the mechanical stimuli into bone formation was investigated and verified in many experimental and clinical studies.

Distraction osteogenesis is currently a standard method of bone lengthening (Choi et al., 2002). In distraction osteogenesis studies, gene expression of IGF-I was detected during distraction (Farhadieh et al., 1999; Yates et al., 2002).

The application of tensile forces to the mid sagittal cranial suture during the growth period cause an increase in the production of IGF-I mRNA and IGF-IR mRNA in osteoblast-like and fibroblastic cells (Hirukaw et al., 2005).

Hajjar et al. (2002) observed that the expression of IGFs increases in condylar cartilage in response to application of propulsive appliances.

In orthodontics, Wescott et al. have applied a 12 % uni-axial cyclic tensile strain to cultured human PDL cells and analyzed the differential expression of 78 genes, 19 genes, including IGF-I, show differential expression. The treated/control (T/C) ratio of IGF-I after 12 hrs was greater than of ± 2 (2.46) but not significant (Wescott et al., 2007). Rath-Deschner et al. have found that IGF-I expression is significantly increased, when cells were subjected to low continuous tensile strain for 4 h (Rath-Deschner et al., 2009). Saggese et al. found a fivefold increase of IGF-I in the cervicular fluid after insertion of an orthodontic appliance to distalize canines in patients aged between 8 and 15 years (Saggese et al., 2005). Toia et al. found that IGF-I increases in the cervicular fluid as early as 4hrs after insertion of fixed appliances (Toia et al., 2005). Therefore, this study indicates that IGF-I and its signals involved in the mechanotransduction processes during the tooth movement.

In this study, the character of IGF-I over time was observed in the PDL and found that IGF-I-positive cells were significantly increased at the tension side in a time dependent manner. It was also recognized that this increase correlated with an increase in the total number of PDL cells over time at the same side. The both IGF-I-positive cells and the total number of the PDL cells reach their peak points after 24 hrs.

It has been observed that the mitotic activity of PDL cells increases markedly on the tension side from 24 to 36 hrs after the initiation of tooth movement (Macapanpan et

al., 1954). This leads to increase the number of periodontal ligament cells, which can be increased by stimulating cell proliferation or by inhibiting apoptosis (Suri and Taneja, 2008). These cell functions can be affected through IGF-I production. IGF-I stimulates proliferation of PDL fibroblast (Palioto et al., 2004), cementoblasts (Grzesik and Narayanan, 2002) and osteoblasts (Wergedal et al., 1990).

The autocrine/paracrine secreted IGF-I is also an important component of the response to injury. Multiple studies in several different animal models have shown that IGF-I is synthesized after injury by the cell types that account for tissue regeneration. This synthesis is necessary for normal tissue repair (Clemmons and Maile 2005), defends against cellular stress and delays the onset of the apoptosis (Kumasheva and Houghton, 2006). The antiapoptotic action of IGFs was recently also observed in PDL cells that respond to IGF-I by up-regulation of anti-apoptotic pathways (Han and Ammar, 2003). Therefore, one can presume that the up-regulation of IGF-I on the tension side can increase the cell number of PDL through its impacts on the proliferation and the apoptosis of the PDL cells during the tooth movement in a time-dependent manner.

The mean values of immunopositive cells for all examined factors reach the peak point after 24 hrs compared with those of the control teeth. These results are in accordance with those described by other researchers for other factors. Transforming growth factor-β1 (TGF-β1) regulates cell proliferation, differentiation, motility and apoptosis (Kanaan and Kanaan, 2006). Osteoprotegerin (OPG) inhibits the differentiation and stimulates the apoptosis of the osteoclasts (Tyrovola et al., 2008). These both factors were observed with significant increase in stretched cells on the bone surface after 24 hrs from force application and became more intense after 48 hrs of force application (Kobayashi et al., 2000).

The expression of epidermal growth factor (EGF) and its receptor, which play an important role in bone formation, were detected in periodontal tissues after 24 hours and 168 hours of tooth movement. The expressions of EGF and EGFR were increased

in periodontal tissues at 168 hrs higher than those at 24 hrs and at tension side higher than those at pressure side at the same time (Gao et al., 2002).

The expression patterns of Ki-67, a cellular marker for proliferation and osteoblast precursors (Scholzen and Gerdes et al., 2000), and Runx2, an essential transcription factor for osteoblast differentiation and chondrocyte maturation (Komori, 2011), were investigated at 3 and 24 hours after appliance insertion. The results showed that the expression of Ki-67 and Runx2 increase in the tension areas after 24 hours of force application (Brooks et al., 2009).

Recently, the gingival crevicular fluid (GCF), which can be collected from the gingival crevice surrounding the teeth, was used to determine possible changes in its constituent in response to various pathological and physiological alterations in the periodontium (Lamster et al., 2007). The presence/expression of regulatory proteins in the GCF has been examined to illustrate the involvement of these proteins in periodontal remodeling provoked by orthodontic stimuli. The most consistent result was a peak of cytokine levels at 24 h (Ren and Vissink, 2008). For example, Nishijima et al., have examined the secretion of RANKL and OPG from hPDL cells at the distal cervical margins of the experimental and control teeth 0, 1, 24, and 168 hrs after the retracting force was applied. After 24 hrs, the levels of RANKL were significantly higher and the levels of OPG were significantly lower compared to the control teeth. There were no such significant differences at 0, 1, or 168 hrs (Nishijima et al., 2006).

All these results support the statement that cellular and tissue reactions, including IGF-I synthesis, start in the initial phase (1-2 days) of tooth movement, immediately after force application. The complex processes of recruitment of osteoclast and osteoblast progenitors begin in this early phase (Krishnan and Davidovitch, 2006).

The human PDL cells represent a heterogeneous cell population including stem cells (Lekic et al., 2001; Silvério et al., 2010) and have the potential to differentiate into various phenotypes, including osteoblasts (Gay et al., 2007) and cementoblasts (Seo et al., 2004). IGF-I induces many differentiated functions, such as production of

collagen and matrix apposition (Grzesik and Narayanan, 2002; Minuto et al., 2005). It is known that procollagen type I is an indicator of the early stage of bone formation. The recombinant IGF-I can raise the serum levels of procollagen type I significantly (Zofkova, 2003). Therefore, it is assumed that IGF-I activates especially the early stages of osteoblastic formation in the PDL.

In periodontal regeneration, the reestablishment of the PDL is required together with corresponding cementum and supporting alveolar bone. Thus, agents that promote PDL cells proliferation and migration as well as collagen biosynthesis would appear to be mediators for enhancing new PDL formation (Raja et al., 2009). Recently, in two in vitro studies, IGF-I alone or in combination with other growth factors promoted osteogenesis of the PDL cells. It can elevate the osteoblastic markers such alkaline phosphatase (ALP) and ostetocalcin (OCN) (Chen et al., 2009; Li et al., 2011). IGFs regulate, in diverse patterns, the differentiation functions of both osteoblasts and osteoclasts (Conover, 2000). This can accelerate the differentiation of osteoclasts and its disappearance in the tension side after force application (disto-coronal areas), which tend to contain more osteoclasts in the PDL of untreated teeth due to the physiological distal drift of the molars in the rats.

Therefore, one can speculate that the up-regulation of IGF-I could influence the differentiation of the PDL cells at the tension side.

As previously mentioned in the introduction, there are four essential interrelated steps in the transduction of mechanical signals by tissues: sensing the mechanical signal by the cells, transduction of this mechanical signal into one that is biochemical, transmission of the biochemical signal to the effector cells, and the effectors cell response (Wise and King, 2008).

After force application both matrix strain and fluid flow in the PDL and the bone cause deformation of cells. Through integrin signalling and other transduction pathways, many mediators are produced leading to activate several types of cells (Hennemann et al., 2008).

Ajubi et al. investigated the signal transduction pathways in osteocytes which were subjected to pulsating fluid flow (PFF). They found that PFF raises intracellular Ca^{2+}. Ca^{2+} and protein kinase C then stimulate phospholipase A2 activity, arachidonic acid production, and ultimately prostaglandin E2 (PGE_2) release (Ajubi et al., 1999; Wise and King, 2008).

Studies which were performed in gingival crevicular fluid (GCF) during tooth movement showed that the concentration of PGE_2 in GCF increased significantly and reaches peak point at 24th hour and generally was higher in tension sides than at the compression sides (Yao et al., 2003; Dudic et al., 2006).

Release of PGE_2 is a prominent load-induced response of osteoblast-like cells. PGE_2 is produced by osteoblasts in response to physiological stress, growth factors, hormones, trauma or inflammatory cytokines and induces cAMP-dependent IGF-I expression by osteoblasts (Papachroni et al., 2009).

In vitro studies showed that PGE_2 increases rapidly at 5min after loading of teeth and led to an increase of intracellular cyclic adenosine monophosphate (cAMP) which peaks at 15 min. (Meikle, 2006).

The increased intracellular cAMP activates protein kinase A (PKA), which stimulates IGF-I gene induction (McCarthy and Centrella, 2001). In addition, mechanical interactions between integrins and their matrix/environment mediate increases in intracellular Ca^{2+} levels and activate mitogen-activated protein (MAP) kinase cascades. This leads to the activation of the activator protein 1 (AP-1) that is necessary for a pro-growth response. The pro-bone growth response involves up-regulation of the genes c-fos, osteocalcin, cyclooxygenase, and IGF-I (Iqbal and Zaidi, 2005).

and thus can man better understand the mechanism of IGF-I up regulation after tooth movement as response to up regulation of other factors such as PGE_2.

In this study, immunoreactivity for IGF-IR was generally more pronounced than that for its ligand IGF-I. The biological actions of IGF-I are predominantly mediated by the IGF-IR (LeRoith 2000; Romano, 2003; Annunziata et al., 2011).

Recently, it has been reported, that growth factor receptors can be also activated by integrins in the absence of the growth factor ligands, due to the interaction between integrins and growth factor receptors (Clemmons and Maile, 2003; Beattie et al., 2010). The crosstalk between integrins and growth factor receptors in multiple cell types may be the result of coclustering of these receptors on the surface of the cell in focal adhesions or in association with the actin cytoskeleton (Eliceiri, 2001, Beattie et al., 2010). For example, the occupation of the αVβ3 integrin receptor with ECM proteins induces IGF-I-stimulated IGF-IR phosphorylation. Conversely, the presence of the αVβ3-specific disintegrin echistatin inhibits the IGF-I-stimulated IGF-IR activation (Kim et al., 2007). In this context, Kapur et al. found that echistatin reduced not only the basal and shear stress-induced TE85 cell (osteosarcoma cell) proliferation but also completely abolished the increase in cell proliferation induced by IGF-I alone as well as that by the combination treatment. This suggests that the synergy between shear stress and IGF-I in osteoblast proliferation involves integrin-dependent recruitment of SHP-2 and -1 away from IGF-IR (Kapur et al., 2005).

Insulin receptor substrates (IRS) is a main target molecule of insulin/IGF-1 receptor signaling and plays important roles in maintaining normal bone turn-over (Ogata and Kawaguchi, 2008). When the concentrations of IRS1 are high, the signal is mitogenic and anti-apoptotic (Valentinis and Baserga, 2001). IRS1 deficient mice exhibited severe low turnover osteopenia with decreased bone anabolic and catabolic action (Akune, 2004).

Tyrosine phosphorylation of IRS1 by the IGF-I receptor tyrosine kinase leads to activation of pathways such PI3 and extracellular signal-regulated kinase 1/2 (ERK1/2). The first pathway protects from apoptosis by blocking the pro-apoptotic proteins and inducing the expression of antiapoptotic proteins. The second pathway, ERK1/2, is required for optimal stimulation of cell division (proliferation) and gene expression (Jones and Clemmons, 1995; Mauro and Surmacz, 2003; Yamagushi et al, 2005). The application of orthodontic loading within the same time interval and under identical experimental circumstances induced a significant increase of ERK1/2-positive PDL cells at the tension side (Kawarizadeh et al., 2005, Pavlidis et al., 2009).

It most kept in mind that ERK1/2 can be activated by other molecules like integrin receptors (Iqbal and Zaidi, 2005; Meikle, 2006).

However, the ability of cells to respond to the autocrine/ paracrine secreted IGF-I is dependent not only on the amount of growth factor that is secreted, but also on the abundance of other proteins in the extracellular environment especially IGFBPs. IGFBPs are a family of secreted proteins that bind IGF with high affinities that are equal to or greater than those of the IGF-IR. In the extracellular environment the most, if not all, of IGFs are bound to specific, high-affinity IGFBPs (Duan and Xu, 2005). They can suppress or enhance the cellular effects of IGFI (Yu and Rohan, 2000; Firth and Baxter, 2002). Anyway, the observed up-regulation of the IGF-IR and IRS1 in this study strengthens the view that the biological action of IGF-I on PDL cells was stimulated under this condition of force application.

At the pressure side, it was observed that all factors are significantly down regulated in the 4 hrs groups, but not in 24 or 48 hrs groups, where the differences between the groups were no more significant. This may be caused by virtue that PDL cells subjected to tensional and compressive forces are able to perceive two different forms of mechanical stimuli and responded in a different manner (He et al., 2004).
Other interpretation for down regulation of IGF-I at the pressure side could due to the negative strain that induced within the PDL at the pressure side of the root (Hennemann et al., 2008) and, consequently, the fibers are relaxed. This leads to unloading of the PDL and alveolar bone (Melsen , 1999, 2001; Binderman et al., 2002). The unloading reduces canalicular fluid flow and induces the apoptosis of osteocytes and osteoblasts, which attracts osteoclasts to the pressure side followed by bone resorption subsequently (Burger et al., 2003; Aguirre et al., 2006; Dufour et al., 2007; Hennemann et al., 2008). Unloading could also inhibit the proliferation and differentiation of osteoprogenitor cells *in vitro* (Kostenuik et al., 1997). Moreover, skeletal unloading induces resistance to IGF-I on bone formation and inhibits the activation of the IGF-I signaling pathway (Sakata et al., 2003, 2004).

Clinically, skeletal unloading during space flight and prolonged bed rest induces osteopenia (Bikle and Hallaoran, 1999).

Thus, one can assume that there is a relation between the unloading of PDL cells, the initial decrease of IGF-I-positive cells, and the decrease of the total cell number at the pressure side.

The positive PDL-cells at the pressure side decreased in the 4 hrs group and augmented subsequently in 24 and 48 hrs groups. This could be interpreted as result of the remodeling activity of PDL tissue and alveolar bone at the pressure side that degrade to create space for the moving tooth while new PDL tissue is simultaneously formed to maintain the attachment (Hennemann et al. 2008).

At the cellular level, PDL cells in the pressure side become a round shape in the first hours of treatment and regain a normal shape 2 or 3 days later (Kirshnan and Davidovitch, 2006). In this regard, cells which are rounded up show catabolic changes whereas flattened cells (under tension) have anabolic effects (Roberts-Harry and Sandy, 2004).

In the present study, the effect of the force magnitude on the positive cells number was only significant between the groups, where 0.1N and 0.5N were applied for 4 hrs and only for IGF-IR. The results for IGF-I and IRS1 showed no significant differences between applied forces but tend to be negative dependent on the force magnitude. These results could indicate a relation between the expression of these molecules, especially IGF-IR, and the magnitude of the applied forces.

In the past 70 years, the concept of optimal force has changed considerably. According to Schwarz, who defined optimal continuous force as ‘the force leading to a change in tissue pressure that approximated the capillary vessels’, forces below the optimal level cause no reaction in the periodontal ligament. Forces exceeding the optimal level would lead to areas of tissue necrosis, preventing frontal bone resorption. Tooth movement would thus be delayed until undermining resorption had eliminated the necrotic tissue obstacle (Schwarz, 1932). Oppenheim and Reitan modified this

definition and advocated the use of the lightest force which is capable to achieve the tooth movement (Oppenheim, 1942; Reitan, 1967). The current concept of optimal force is based on the hypothesis, that a force of a certain magnitude and temporal characteristics would be capable of producing a maximum rate of tooth movement without tissue damage and with maximum patient comfort. The optimal force for tooth movement may differ for each tooth and for each individual patient (Ren et al., 2003).

There are many reports about the effect of force magnitude on tooth movement. King et al. demonstrated that the effective tooth movement of rat molars ranges from 20 g to 40 g and its velocity does not increase over 40 g (King et al., 1991). Gonzales et al. found that the light forces produce more tooth movement and less root resorption compared with heavier forces (Gonzales et al., 2007).

Considering the orthodontic tooth movement as the result of biological reactions to externally applied mechanical stimuli, the application of 0.1 N may cause greater biologic activity of IGF-I-signalling than the other forces, which can subsequently lead to cellular responses aiming at adaptation to the applied forces.

7. Summary

The aim of this study was to characterize the role of IGF-I and its signalling *in vivo* as an early reaction to the mechanotransduction process, and to analyse the changes of the local expression of these factors related to the magnitude of the applied force.

The right upper first molars of 20 anesthetised rats were loaded with forces in order to be moved mesially. In the first group of animals, constant forces for 4 hours of 0.1 N, 0.25 N and 0.5 N were applied in four animals each. The applied forces were kept constant and recorded continuously for 4 hrs. After 4 hrs all 12 animals were sacrificed. In the second group, constant forces of 0.1 N were applied for 2 hours in 8 animals. Afterwards, the first and second molars in the second group were passively separated with composite and the occlusal contacts were eliminated by slicing the antagonistic lower molars. Four rats were sacrificed after 24 hrs and four after 48 hrs. The untreated contralateral molars in 4 rats served as controls. Paraffin-embedded sections of the resected jaws were prepared for immunohistochemistry to localize insulin-like growth factor-I (IGFI), its receptor (IGF-IR), and insulin receptor substrate 1 (IRS1). Histomorphometric analysis was performed to count the percentage of immunoreactive cells in different parts of the periodontal ligament. The statistical analyses were performed with SPSS Software.

The results showed that IGF-I-, IGF-IR- and IRS1-positive cells were observed in the periodontal tissues of the control and loaded teeth. In the experimental group, the number of IGF-I-, IGF-IR- and IRS1-positive cells increased significantly on the tension side and decreased significantly on the compression side. The increase of immunopositive cells at the tension side was in a time-dependent manner and tended to be dependent on the force magnitude. The decrease in the immunopositive cells at the pressure side was only observed in the first group (4 hrs) and tended to be dependent on the force magnitude.

These data indicate a close relationship between mechanical loading of the PDL and the autocrine/paracrine expression of IGF components as an early step in the

mechanotransduction process leading to an organized remodelling of the PDL and alveolar bone in the long term.

Moreover, the local increase/decrease of IGF-I appears to provide a link between the mechanical loading/unloading, the activation/inhibition of proliferation and differentiation of PDL cells and the inhibition/induction of the antiapoptotic activity in the periodontal ligament in the early phase of tooth movement. However, more studies are required to confirm or rule out these hypotheses.

8. References

Adams GR. Autocrine/paracrine IGF-I and skeletal muscle adaptation. J Appl Physiol. 2002; 93: 1159-1167

Aguirre JI, Plotkin LI, Stewart SA, Weinstein RS, Parfitt AM, Manolagas SC, Bellido T. Osteocyte apoptosis is induced by weightlessness in mice and precedes osteoclast recruitment and bone loss. J Bone Miner Res. 2006; 21: 605-615

Akune T. The role of insulin receptor substrates in bone metabolism. Clin Calcium. 2004; 14: 289-292

Ajubi NE, Klein-Nulend J, Alblas MJ, Burger EH, Nijweide PJ. Signal transduction pathways involved in fluid flow-induced PGE2 production by cultured osteocytes. Am J Physiol. 1999; 276: 171-178

Annunziata M, Granata R, Ghigo E. The IGF system. Acta Diabetol. 2011; 48: 1-9

Barton ER, Crowder C. Growth Factor Targets for Orthodontic Treatments. Semin Orthod. 2010; 16: 128-134

Baumrind S. A reconsideration of the property of the pressure tension hypothesis. Am J Orthod. 1969; 55: 12-22

Beattie J, McIntosh L, van der Walle CF. Cross-talk between the insulin-like growth factor (IGF) axis and membrane integrins to regulate cell physiology. J Cell Physiol. 2010; 224: 605-611

Bikle DD, Hallaoran BP. The response of bone to unloading. J Bone Miner Metab. 1999; 17: 233–244

Binderman I, Bahar H, Yaffe A. Strain relaxation of fibroblasts in the marginal periodontium is the common trigger for alveolar bone resorption: a novel hypothesis. J Periodontol. 2002; 73: 1210-1215

Bonapace G, Concolino D, Formicola S, Strisciuglio P. A novel mutation in a patient with Insulin-like growth factor 1 (IGF1) deficiency. J Med Genet. 2003; 40: 913-917

Bouletreau PJ, Warren SM, Longaker MT. The molecular biology of distraction

osteogenesis. J Craniomaxillofac Surg. 2002; 30: 1-11

Brooks PJ, Nilforoushan D, Manolson MF, Simmons CA, Gong SG. Molecular markers of early orthodontic tooth movement. Angle Orthod. 2009; 79:1108-1113.

Butler A, LE Roith D. Minireview: Tissue-specfic versus generalized gene targeting of the igf1 and igf1r genes and their roles in insulin-like growth factor physiology. Endocrinology 2001; 142:1685-1688

Burger EH, Klein-Nulend J, Smit TH. Strain-derived canalicular fluid flow regulates osteoclast activity in a remodelling osteon a proposal. J Biomech. 2003; 36:1453-1459.

Burstone CJ. The biomechanics of tooth movement. In: Kraus BS, Riedel RA, editors. Vistas in orthodontics. Philadelphia: Lea and Febiger; 1962

Butt AJ, Williams AC. IGFBP-3 and apoptosis-a licence to kill? Apoptosis 2001; 6: 199–205

Canalis E. Growth factor control of bone mass. J Cell Biochem. 2009; 108: 769-777

Chachisvilis M, Zhang YL, Frangos JA. G protein-coupled receptorssense fluid shear stress in endothelial cells. Proc Natl Acad Sci USA. 2006; 103: 15463-15468

Chen FM, Chen R, Wang XJ, Sun HH, Wu ZF. In vitro cellular responses to scaffolds containing two micro encapulated growth factors. Biomaterials 2009; 30: 5215-5224

Choi IH, Chung CY, Cho TJ, Yoo WJ. Angiogenesis and mineralization during distraction osteogenesis. J Korean Med Sci. 2002; 17: 435-447

Clemmons DR, Maile LA. Interaction between Insulin-like growth factor-I receptor and Vß3 integrin linked signaling pathways: cellular responses to changes in multiple signaling inputs. Mol Endocrinol. 2005; 19: 1-11

Cohen P. Overview of the IGF-I system. Horm Res. 2006; 65: 3–8

Conover CA. *In vitro* studies of insulin-like growth factor I and bone. Growth Horm IGF Res. 2000; 10: 107–110

Davidson RM, Tatakis DW, Auerbach AL. Multiple forms of mechanosensitive

channels in osteoblast-like cells. Pflugers Archiv. 1990; 416: 646-651

DeChiara TM, Robertson EJ, Efstratiadis A. Parental imprinting of the mouse insulin-like growth factor II gene. Cell 1991; 64: 849–859

Denley A, Cosgrove LJ, Booker GW, Wallace JC, Forbes BE. Molecular interactions of the IGF system. Cytokine Growth Factor Rev. 2005; 16: 421-439

Duan C, Xu Q. Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. Gen Comp Endocrinol. 2005; 142: 44-52

Dudic A, Kiliaridis S, Mombelli A, Giannopoulou C. Composition changes in gingival crevicular fluid during orthodontic tooth movement: comparisons between tension and compression sides. Eur J Oral Sci. 2006; 114: 416-422

Dufour C, Holy X, Marie PJ. Skeletal unloading induces osteoblast apoptosis and targets alpha5beta1-PI3K-Bcl-2 signaling in rat bone. Exp Cell Res. 2007; 15:394-403.

Duncan R L, Turner C H. Mechanotransduction and the functional response of bone to mechanical strain. Calcif Tissue Int. 1995; 57: 344-358

Dupont J, Holzenberger M. Biology of insulin-like growth factors in development. Birth Defects Res C Embryo Today. 2003; 69: 257-271

Eliceiri BP. Integrin and growth factor receptor crosstalk. Circ Res. 2001; 89: 1104-1110

Farhadieh RD, Dickinson R, YU Y, Gianutsos MP, Walsh WR**.** The role of transforming growth factor, insulin like growth factor I, and basic fibroblast growth factor in distraction osteogenesis of the mandible. J Craniofac Surg. 1999; 10: 80-86

Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev. 2002; 23: 824–854

Frystyk J. Exercise and the growth hormone-insulin-like growth factor axis. Med Sci Sports Exerc. 2010; 42: 58-66

Fujiwara N, Tabata MJ, Endoh M, Ishizeki K, Nawa T. insulin-like growth factor-I stimulates cell proliferation in the outer layer of Hertwig's epithelial root sheath and

elongation of the tooth root in mouse molars *in vitro*. Cell Tissue Res. 2005; 320: 69-75

Gay IC, Chen S, MacDougall M. Isolation and characterization of multipotent human periodontal ligament stem cells. Orthod Craniofac Res. 2007; 10: 149–160

Gao Q, Zhang S, Jian X, Zeng Q, Ren L. Expression of epidermal growth factor and epidermal growth factor receptor in rat periodontal tissues during orthodontic tooth movement. Zhonghua Kou Qiang Yi Xue Za Zhi 2002; 37: 294-296

Goldspink G. Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. J Anat. 1999; 194: 323–334

Götz W, Krüger U, Ragotzki S, Lossdörfer S, Jäger A. Immunohistochemical localization of components of insulin-like growth factor-system in human decidous teeth. Connect Tissue Res. 2001; 42: 291-302

Götz W, Lossdörfer S, Krüger U, Braumann B, Jäger A. Immunhistochemical localization of insulin-like growth factor-II and its binding protein-6 in human epithelial cells of Malassez. Eur J Oral Sci. 2003; 11: 26-33

Götz W, Heinen M, Lossdörfer S, Jäger A. Immunohistochemical localization of components of insulin-like growth factor-system in human permanent teeth. Arch Oral Biol. 2006(a); 51: 387-395

Götz W, Kunert D, Zhang D, Kawarizadeh A, Lossdörfer S, Jäger A. Insulin-like growth factor system components in the periodontium during tooth root resorption and early repair processes in the rat. Eur J Oral Sci. 2006 (b);114: 318-327

Govoni E, Baylink DJ, Mohan S. The multi-functional role of insulin-like growth factor binding proteins in bone. Pediatr Nephrol. 2005; 20: 261-268

Grimm FM. Bone bending, a feature of orthodontic tooth movement. Am J Orthod 1972; 62: 384-393

Grzesik WJ, Narayanan AS. Cementum and periodontal wound healing and regeneration. Crit Rev Oral Biol Med. 2002, 13: 474-484

Hajjar D, Santos MF, Kimura IT. Propulsive appliance stimulates the synthesis of insulin-like growth factors I and II in the mandibular condylar cartilage of young rats. Arch Oral Biol. 2003; 48: 635-642

Han X, Amar S. IGF-1 signaling enhances cell survival in periodontal ligament fibroblasts vs. gingival fibroblasts. J Dent Res. 2003; 82: 454-459

Hamill OP, Martinac B. Molecular basis of mechanotransduction in living cells. Physiol Rev. 2001; 81: 685-740

Harell A, Dekel S, Binderman I. Biochemical effect of mechanical stress on cultured bone cells. Calcif Tissue Res. 1977; 22: 202–207

Haut Donahue TL, Genetos DC, Jacobs CR, Donahue HJ, Yellowley CE. Annexin V disruption impairs mechanically induced calcium signaling in osteoblastic cells. Bone 2004; 35: 656–663

He Y, Macarak EJ, Korostoff JM, Howard PS. Compression and tension: differential effects on matrix accumulation by periodontal ligament fibroblasts *in vitro*. Connect Tissue Res. 2004; 45: 28–39

Henneman S, Von den Hoff JW, Maltha JC. Mechanobiology of tooth movement. Eur J Orthod. 2008; 30: 299-306

Hirukaw K, Miyazawa K, Maeda H, Kameyama Y, Goto S, Togari A. Effect of tensile force on the expression of IGF-I and IGF-I receptor in the organ-cultured rat cranial suture. Arch Oral Biol. 2005; 50: 367-372

Holly J, Perks C. The role of insulin-like growth factor binding proteins. Neuroendocrinology 2006; 83: 154-160

Iqbal J, Zaidi M. Molecular regulation of mechanotransduction. Biochem Biophys Res Commun. 2005; 328: 751-755

Iwasaki LR, Crouch LD, Nickel JC. Genetic factors and tooth movement. Semin Orthod. 2008; 14: 135-145

Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev. 1995; 16: 3-34

Kanaan RA, Kanaan LA. Transforming growth factor beta1, bone connection. Med Sci Monit. 2006; 12: 164-169

Kapur S, Mohan S, Baylink DJ, Lau KH. Fluid shear stress synergizes with insulin-like growth factor-I (IGF-I) on osteoblast proliferation through integrin-dependent activation of IGF-I mitogenic signaling pathway. J Biol Chem. 2005; 280: 20163-20170

Kawarizadeh A, Bourauel C, Götz W, Jäger A. Early responses of periodontal ligament cells to mechanical stimulus *in vivo*. J Dent Res 2005; 84: 902-906

Kelley KM, Schmidt KE, Berg L, Sak K, Galima MM, Gillespie C, Balogh L, Hawayek A, Reyes JA, Jamison M. Comparative endocrinology of the insulin-like growth factor-binding proteins. J Endocrinol. 2002; 175: 3-18

Kim SK, Kwon JY, Nam TJ. Involvement of ligand occupancy in Insulin-like growth factor-I (IGF-I) induced cell growth in osteoblast like MC3T3-E1 cells. Biofactors 2007; 29: 187-202

Kjaer M, Langberg H, Heinemeier K, Bayer ML, Hansen M, Holm L, Doessing S, Kongsgaard M, Krogsgaard MR, Magnusson SP. From mechanical loading to collagen synthesis, structural changes and function in human tendon. Scand J Med Sci Sports. 2009; 19: 500-510

Klein-Nulend J, Semeins CM, Ajubi NE, Nijweide PJ, Burger EH. Pulsating fluid flow increases nitric oxide (NO) synthesis by osteocytes but not periosteal fibroblasts correlation with prostaglandin upregulation. Biochem Biophys Res Commun. 1995;

217: 640–648

Klein-Nulend J, Bacabac RG, Mullender MG. Mechanobiology of bone tissue. Pathol Biol. 2005; 53: 576-580

Kobayashi Y, Hashimoto F, Miyamoto H, Kanaoka K, Miyazaki-Kawashita Y, Nakashima T, Shibata M, Kobayashi K, Kato Y, Sakai H. Force-induced osteoclast apoptosis in vivo is accompanied by elevation in transforming growth factor beta and osteoprotegerin expression. J Bone Miner Res. 2000; 15: 1924-1934

Kolahi KS, Mofrad MR. Mechanotransduction: a major regulator of homeostasis and development. Wiley Interdiscip Rev Syst Biol Med. 2010; 2: 625-639

Kostenuik PJ, Halloran BP, Morey-Halton ER, Bikle DD. Skeletal unloading inhibits the *in vitro* poliferation and differentiation of rat osteoprogenitor cells. Am J Physiol. 1997; 273: 1133-1139

Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofacial Orthop. 2006; 129: 1-32

Krishnan V, Davidovitch Z. On a path to unfolding the biological mechanisms of orthodontic tooth movement. J Dent Res. 2009; 88: 597-608

Kumasheva RT, Houghton PT. IGF-I mediated survival pathways in normal and malignant cells. Biochim Biophys Acta. 2006; 1766: 1-22

Lamster IB, Ahlo JK. Analysis of gingival crevicular fluid as applied to the diagnosis of oral and systemic diseases. Ann N Y Acad Sci. 2007; 1098: 216-229

Laron Z, Klinger B, Silbergeld A. Patients with Laron syndrome have osteopenia/ osteoporosis. J Bone Miner Res. 1998; 14: 156–157

Laron Z. Insulin like growth factor 1 (IGF-1): a growth hormone. J Clin Pathol. 2001; 54: 311-316

Laviola L, Natalicchio A, Giorgino F. The IGF-I signaling pathway. Curr Pharm Des. 2007; 13: 663-669

Lekic P, Rojas J, Birek C, Tenenbaum H, McCulloch CA. Phenotypic comparison of periodontal ligament cells *in vivo* and *in vitro*. J Periodontal Res. 2001; 36: 71-79

Legate KR, Wickström SA, Fässler R. Genetic and cell biological analysis of integrin outside-in signaling. Genes Dev. 2009; 23: 397-418

LeRoith D. Insulin-like growth factor I receptor signaling-overlapping or redundant pathways? Endocrinology. 2000; 141: 1287-1288

LE Roith D, Bondy C, Yakar S, Liu J L, Butler A. The somatomedin hypothesis. Endocr Rev. 2001; 22: 53-74

Lelbach A, Muzes G, Feher J. The insulin-like growth factor system: IGFs, IGF-binding proteins and IGFBP-proteases. Acta Physiol Hung. 2005; 92: 97-107

Li Y, Niu ZY, Tang CH, Si SY. Effect of insulin-like growth factor on the proliferation and early stage osteogenesis of human periodontal ligament stem cells under three-dimensional culture system. Zhonghua Kou Qiang Yi Xue Za Zhi 2011; 46: 143-147

Liedert A, Kaspar D, Augat P, Ignatius A, Claes L. Mechanobiology of Bone Tissue and Bone Cells. In: Kamkin A, Kiseleva I, editors. Mechanosensitivity in Cells and Tissues. Moscow: Academia; 2005

Liu W, Bloom DA, Cance WG, Kurenova EV, Golubovskaya VM, Hochwald SN. FAK and IGF-IR interact to provide survival signals in human pancreatic adenocarcinoma cells. Carcinogenesis 2008; 29: 1096–1107

Macapanpan L, Weinmann J, Brodie A. Early tissue changes following tooth movement in rats. Angle Orthod. 1954; 24: 79–95

Masella RS, Meister M. Current concepts in the biology of orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 2006; 129: 458-468

Mauro L and Surmacz E. IGF-I receptor, cell-cell adhesion, tumor development and progression. J Mol Histol. 2004; 35: 247-253

McCarthy T L, Centrella M. Local IGF-I expression and bone formation. Growth Horm IGF Res. 2001; 11: 213–219

McDonald F, Somasundaram B, McCann TJ, Mason WT, Meikle MC. Calcium waves in fluid flow stimulated osteoblasts are G protein mediated. Arch Biochem Biophys. 1996; 326: 31-38

Meikle MC. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. Eur J Orthod. 2006; 28: 221-240

Melsen B. Biological reaction of alveolar bone to orthodontic tooth movement. Angle Orthod. 1999; 69: 151-158

Melsen B. Tissue reaction to orthodontic tooth movement: a new paradigm. Eur J Orthod. 2001; 23: 671-681

Mohan S, Baylink D. Bone growth factors. Clin Orthop Relat Res. 1991; 263:30-48.

Mohan S, Baylink D. IGF-binding proteins are multifunctional and act via IGF-dependent and -independent mechanisms. J Endocrinol. 2002; 175: 19-31

Myers KA, Rattner JB, Shrive NG, Hart DA. Osteoblast-like cells and fluid flow: cytoskeletondependent shear sensitivity. Biochem Biophys Res Commun. 2007; 364: 214–219

Netchine I, Azzi S, Le Bouc Y, Savage MO. IGF1 molecular anomalies demonstrate its critical role in fetal, postnatal growth and brain development. Best Pract Res Clin Endocrinol Metab. 2011; 25: 181-190

Nishijima Y, Yamaguchi M, Kojima T, Aihara N, Nakajima R, Kasai K. Levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement and effect of compression force on releases from periodontal ligament cells *in vitro*. Orthod Craniofac Res. 2006; 9: 63-70

Ogata N, Chikazu D, Kubota N, Terauchi Y, Tobe K, Azuma Y, Ohta T, Kadowaki T, Nakamura K, Kawaguchi H. Insulin receptor substrate-1 in Osteoblast is indispensable for maintaining bone turnover. J Clin Invest. 2000; 105: 935-943

Ogata N, Kawaguchi H. Involvement of insulin and IGF-1 signaling molecules in bone metabolism. Clin Calcium. 2008; 18: 614-622

Ohlsson C, Sjögren K, Jansson JO, Isaksson OG. The relative importance of endocrine versus autocrine/paracrine insulin-like growth factor-I in the regulation of body growth Pediatr Nephrol. 2000; 14: 541-543

Olesen JL, Heinemeier KM, Haddad F, Langberg H, Flyvbjerg A, Kjaer M, Baldwin KM. Expression of insulin-like growth factor I, insulin-like growth factor binding proteins, and collagen mRNA in mechanically loaded plantaris tendon. J Appl Physiol. 2006; 101: 183-188

Oppenheim A. Tissue changes, particularly of the bone, incident to tooth movement. Am Orthod. 1911; 3: 57-67

Oppenheim A. Human tissue response to orthodontic intervention of short and long duration. Am J Orthod Oral Surg. 1942; 28: 263–301

Palioto DB, Coletta RD, Graner E, Joly JC, de Lima AF. The influence of enamel matrix derivative associated with insulin-like growth factor-I on periodontal ligament fibroblasts. J Periodont. 2004; 75: 498-504

Papachroni KK, Karatzas DN, Papavassiliou KA, Basdra EK, Papavassiliou AG. Mechanotransduction in osteoblast regulation and bone disease. Trends Mol Med. 2009; 15: 208-216

Pavelić J, Matijević T, Knezević J. Biological and physiological aspects of action of insulin-like growth factor peptide family. Indian J Med Res. 2007; 125: 511-522

Pavlidis D, Bourauel C, Rahimi A, Götz W, Jäger A. Proliferation and differentiation of periodontal ligament cells following short-term tooth movement in the rat using different regimens of loading. Eur J Orthod. 2009; 31: 565-571

Pavlin D, Gluhak-Heinrich J. Effect of mechanical laodind on periodontal cells. Crit Rev Oral Biol Med. 2001; 12: 414-424

Pilon AM, Kuijpers-Jagtman AM, Maltha JC. Magnitude of orthodontic forces and rate of bodily tooth movement: an experimental study in beagle dogs. Am J Orthod Dentofacial Orthop. 1996; 110: 16-23

Proffit WR. Biologic basis of orthodontic therapy. In: Proffit WR, Fields jr HW, Sarver DM (eds). Contemporary orthodontics. 4th ed. Mosby-Elsevier; St. Louis 2007, 331-358

Quinn RS, Yoshikawa K. A reassessment of force magnitude in orthodontics. Am J Orthod. 1985; 88: 252–260

Raja S, Byakod G, Pudakalkatti P. Growth factors in periodontal regeneration. Int J Dent Hyg. 2009; 7: 82-89

Rath-Deschner B, Deschner J, Reimann S, Jäger A, Götz W. Regulatory effects of biomechanical strain on the insulin-like growth factor system in human periodontal cells. J Biomech. 2009; 42: 2584-2589

Reijnders CM, Bravenboer N, Tromp AM, Blankenstein MA, Lips P. Effect of mechanical loading on insulin-like growth factor-I gene expression in rat tibia. J Endocrinol. 2007; 192: 131-140

Reitan K. Clinical and histologic observations on tooth movement during and after orthodontic treatment. Am J Orthod. 1967; 53: 721–745

Ren Y, Maltha JC, Kuijpers-Jagtman AM. Optimum force magnitude for orthodontic tooth movement: a systematic literature review. Angle Orthod. 2003; 73: 86-92

Ren Y, Vissink A. Cytokines in crevicular fluid and orthodontic tooth movement. Eur J Oral Sci. 2008; 116: 89-97

Roberts-Harry D, Sandy J. Orthodontics. Part 11: Orthodontic tooth movement. Br Dent J. 2004; 196: 391-394

Romano G. The complex biology of the receptor for the insulin-like growth factor-1. Drug News Perspect. 2003; 16: 225-231

Rosen CJ. Serum Insulin-like growth factors and Insulin-like growth factors-binding Proteins: clinical implications. Clin Chem. 1999; 45: 1384-1390

Saggese R, Federico G, Gandini P. The IGF-1-IGFBPs system in the crevicular fluid: its changes during orthodontic movement. Prog Orthod. 2005; 6: 114-118

Sakata T, Halloran BP, Elalieh HZ, Munson SJ, Rudner L, Venton L, Ginzinger D, Rosen CJ, Bikle DD. Skeletal unloading induces resistance to insulin-like growth factor I on bone formation. Bone 2003; 32: 669-680

Sakata T, Wang Y, Halloran BP, Elalieh HZ, Cao J, Bikle DD. Skeletal unloading induces resistance to Insulin-like growth factor-I (IGF-I) by inhibiting activation of the IGF-I signalling pathways. J Bone Miner Res. 2004; 19: 436-446

Samani AA, Yakar S, LeRoith D, Brodt P. The role of the IGF system in cancer growth and metastasis: overview and recent insights. Endocr Rev. 2007; 28: 20-47

Sandy JR, Meghji S, Farndale RW, Meikle MC. Dual elevation of cyclic AMP and inositol phosphates in response to mechanical deformation of murine osteoblasts. Biochim Biophys Acta. 1989; 1010: 265–169

Sandy JR. Signal transduction. Br J Orthod. 1998; 25: 269-274

Sandstedt C. Einige Beiträge zur Theorie der Zahnregulierung. Nord Tandlaeg Tidskr. 1904; 5: 236-256

Sant'Ana AC, Marques MM, Barroso TE, Passanezi E, de Rezende ML. Effects of TGF-beta1, PDGF-BB, and IGF-1 on the rate of proliferation and adhesion of a periodontal ligament cell lineage *in vitro*. J Periodontol. 2007; 78: 2007-2017

Sawakami K, Robling AG, Ai M, Pitner ND, Liu D, Warden SJ, Li J, Maye P, Rowe DW, Duncan RL, Warman ML, Turner CH. The Wnt co-receptor LRP5 is essential for skeletal mechanotransduction but not for the anabolic bone response to parathyroid hormone treatment. J Biol Chem. 2006*;* 281: 23698-23711

Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol. 2000; 182: 311–322

Schwarz AM. Tissue changes incident to orthodontic tooth movement. Int J Orthod. 1932; 18: 331-352

Schwartz MA. Integrins and extracellular matrix in mechanotransduction. Cold Spring Harb Perspect Biol. 2010; 2: a005066

Scott A, Khan KM, Duronio V, Hart DA. Mechanotransduction in human bone: *in vitro* cellular physiology that underpins bone changes with exercise. Sports Med. 2008; 38: 139–160

Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004; 364: 149-155

Silvério KG, Rodrigues TL, Coletta RD, Benevides L, Da Silva JS, Casati MZ, Sallum EA, Nociti FH Jr. Mesenchymal stem cell properties of periodontal ligament cells from deciduous and permanent teeth. J Periodontol. 2010; 81: 1207-1215

Storey E, Smith R. Force in orthodontics and its relation to tooth movement. Aust Dent J. 1952; 56: 11–18

Suri L and Taneja P. Surgically assisted rapid palatal expansion: A literature review. AJO. 2008; 133: 290-302

Termsuknirandorn S, Hosomichi J, Soma K. Occlusal stimuli influence on the expression of IGF-1 and the IGF-1 receptor in the rat periodontal ligament. Angle Orthod. 2008; 78: 610-616

Toia M, Galazzo R, Maioli C, Granata R, Scarlatti F. The IGF-I/IGFBP-3 system in gingival crevicular fluid and dependence on application of fixed force. J Endocrinol Invest. 2005; 28: 1009–1014

Tyrovola JB, Spyropoulos MN, Makou M, Perrea D Root resorption and the OPG/RANKL/RANK system: a mini review. J Oral Sci. 2008; 50: 367-376

Vas Leeuwen EJ, Maltha JC, Kuijpers-Jagtsman AM. Tooth movement with light continuous and discontinuous forces in beagle dogs. Eur J Oral Sci. 1999; 107: 468-474

Valentinis B, Baserga R. IGF-I receptor signalling in transformation and differentiation. Mol Pathol. 2001; 54: 133-137

von Böhl M, Maltha JC, Von Den Hoff JW, Kuijpers-Jagtman AM. Focal hyalinization during experimental tooth movement in beagle dogs. Am J Orthod Dentofacial Orthop. 2004; 125: 615-623

von Böhl M, Kuijpers-Jagtman AM. Hyalinization during orthodontic tooth movement: a systematic review on tissue reactions. Eur J Orthod. 2009; 31: 30-36

Waddington RJ, Embery G. Proteoglycans and orthodontic tooth movement. J Orthod. 2001; 28: 281-290

Wang JH, Thampatty BP. An introductory review of cell mechanobiology. Biomech Model Mechanobiol. 2006; 5: 1–16

Wergedal JE, Mohan S, Lundy M, Baylink DJ. Skeletal growth factor and other growth factors known to be present in bone matrix stimulate proliferation and protein synthesis in human bone cells. J Bone Miner Res. 1990; 5: 179-186

Werner H, Katz J. The emerging role of the Insulin-like growth factors in oral biology. J Dent Res. 2004; 83: 832-836

Wescott DC, Pinkerton MN, Gaffey BJ, Beggs KT, Milne TJ and Meikle MC. Osteogenic Gene Expression by Human Periodontal Ligament Cells under Cyclic Tension. J Dent Res. 2007; 86: 1212-1216

Wise GE, King GJ. Mechanisms of tooth eruption and orthodontic tooth movement. J Dent Res. 2008; 87: 414-434

Wolf E, Rapp K, Blum WF, Kolb H, Brem G. Skeletal growth of transgenic mice with elevated levels of circulating insulin-like growth factor-II. Growth Regul. 1995; 5: 177–183

Woods KA, Camacho-Hubner C, Savage MO, Clark AJ. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med. 1996; 335: 1363–1367

Xian C, Wang Y, Zhang B, Tang L. Effects of mechanical strain on the proliferation and expression of IGF-1 mRNA in rat osteoblasts. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 2007; 24: 312-315

Yakar S, Rosen CJ. From mouse to man: redefining the role of insulin-like growth factor-I in the acquisition of bone mass. Exp Biol Med. 2003; 228: 245-252

Yamaguchi M, Ogata N, Shinoda Y, Akune T, Kamekura S, Terauchi Y, Kadowaki T, Hoshi K, Chung U, Nakamura K, Kawaguch H. Insulin Receptor Substrate-1 Is Required for Bone Anabolic Function of Parathyroid Hormone in Mice. Endocrinology 2005; 146: 2620-2628

Yao YL, Feng XP, Jing XZ. The correlation between tooth pain and bioactivators changes in gingival crevicular fluid after applying orthodontic stress. Shanghai Kou Qiang Yi Xue 2003; 12: 331–333

Yates KE, Troulis MJ, Kaban LB, Glowacki J. IGF-I, TGF-II, and BMP-4 are expressed during distraction osteogenesis of the pig mandible. Int J Oral Maxillofac Surg. 2002; 31: 173-178

Yeh CK, Rodan GA. Tensile forces enhance prostaglandin E2 synthesis in osteoblastic cells grown on collagen ribbons. Calcif Tissue Int. 1984; 36: 67–71

Yu H, Rohan T. Role of the Insulin-like growth factor family in cancer development and progression. JNCI. 2000; 92: 1472-1489

Zhao G, Monier-Faugere MC, Langub MC, Geng Z, Nakayama T, Pike JW, Chernausek SD, Rosen CJ, Donahue LR, Malluche HH, Fagin JA, Clemens TL. Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: increased trabecular bone volume without increased osteoblast proliferation. Endocrinology 2000; 141: 2674-2682

Zofkova I. Pathophysiological and clinical importance of Insulin-like growth factor-1 with respect to bone metabolism. Physiol Res. 2003; 52: 657-679

9. Danksagung

An erster Stelle danke ich meinem Doktorvater Herrn Prof. Dr. med. Werner Götz für die Überlassung des interessanten Dissertationsthemas, die Unterstützung und Diskussionsbereitschaft. Durch seine hervorragende Betreuung und sein kontinuierliches Interesse am Fortgang dieser Arbeit fühlte ich mich stets optimal betreut.

Mein ganz besonderer Dank gilt Herrn Prof. Dr. med. dent andreas Jäger ganz herzlich, weil er mir die Möglichkeit gegeben hat, die Fachausbildung der Kieferorthopädie unter seiner Aufsicht machen zu können und diese Dissertation in der kieferorthopädischen Abteilung der Zahnklinik in Bonn schreiben zu können.

Herrn Prof. Dr. Christoph Bourauel danke ich auch für die Entwicklung des Versuchsaufbaus.

Ich danke ebenfalls Frau MTA Inka Bay-Müller für die sorgfältige Einweisung in die immunhistochemische Laborarbeit.

Frau Dr. Klarmann aus dem Institut für Biometrie danke ich für die geduldige und freundliche Einarbeitung in das Statistikprogramm SPSS.
Des Weiteren danke ich der Universität Aleppo für die finanzielle Unterstützung meines Aufenthalts in Deutschland.
Diese Studie war Teil der geplanten Untersuchungen des Teilprojektes 7 der klinischen Forschungsgruppe 208.
Schließlich möchte ich mich bei meinen Eltern und meiner Frau bedanken. Ohne die liebevolle Unterstützung von ihnen wäre diese Arbeit in der erlebten Art und Weise nicht möglich gewesen.