T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF ORTHODONTICS

# EFFECTS OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS ON BONE FORMATION AND STABILITY OF ORTHODONTIC MINI SCREW IMPLANTS

DOCTOR OF PHILOSOPHY THESIS

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APPROVAL

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## DECLARATION

I hereby declare that this thesis is my own work, I have obeyed all the ethical rules in every stage of preparation of my thesis, and to the best of my knowledge and belief, it contains neither material previously published or written by another person, nor material which had been accepted for the award of any other degree; except where explicit reference is mentioned in the text.

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Nasim Mesgarzadeh

## DEDICATION

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## LIST OF SYMBOLS AND ABBREVIATIONS

**3D** Three-Dimensional

**ALP** Alkaline Phosphatases

**ASCs** Adipose-derived Stromal/Stem Cells

**BMP** Bone Morphogenetic Protein

**BMSCs** Bone Marrow Stromal/Stem Cells

**BS** Bone Surface

**BV/TV** Bone Volume/Tissue Volume

**CD** Cluster of Differentiation

**CT** Computed Tomography

**DAPI** 49,6- diamidino-2-phenylindole

**hASCs** Human Adipose-Derived Stromal/Stem Cells

**cN** centi Newton

**ECM** Extracellular Matrix

**ERK** Extracellular signal-Related Kinase

**EU** European Union

**FDA** Food and Drug Administration

**FGF** Fibroblast Growth Factor

**FMA** Frankfort-Mandibular plane Angle

**FN** Fibronectin

**hTGSCs** human Tooth Germ Stem Cells

**HU** Hounseld Units

**IGF** Insulin-like Growth Factor

**IL** Interleukins

**ISQ** Implant Stability Quotient

**LED** Light-Emitting Diode

**LPT** Photobiomodulation Therapy

**MIT** Maximum Insertion Torque

**MSCs** Mesenchymal Stromal/Stem Cells

**SD** Standard Deviation

**MSIs** Mini Screw Implants

**MVs** Membrane-derived Vesicles

**NaB** sodium pentaborate pentahydrate

**NSAID** NonSteroidal Anti-Inflammatory Drug

**OCL** Osteocalcin

**ON** Osteonectin

**OPN** Osteopontin

**PDGF** Platelet-Derived Growth Factor

**PPAR** Peroxisome Proliferator-Activated Receptor

**PRGF** Platelet-Released Growth Factor

**PRP** Platelet-Rich Plasma

**PTV** Periotest Value

**RANKL** Receptor Activator of Nuclear factor κ-B Ligand

**SPSS** Statistical Package for Social Sciences

**SVF** Stromal Vascular Fraction

**Tb.N** Trabecular Number

**Tb.Sp** Trabecular Separation

**Tb.Th** Trabecular Thickness

**TCP** Tricalcium Phosphate

**TGF** Transforming Growth Factor

**TNF** Tumor Necrosis Factor

**VEGF** Vascular Endothelial Growth Factor

**µCT** micro-Computed Tomography

## ABSTRACT

**Mesgarzadeh, N. (2018). Effects of adipose-derived mesenchymal stem cells on bone formation and stability of orthodontic mini screw implants. Yeditepe University Institute of Health Sciences, Orthodontics PhD Thesis, Istanbul.**

The aim of this randomized controlled experimental animal study was to evaluate the differences between the stability of Stem cell (SC)- treated orthodontic mini screw Implants (MSIs); with or without Boron versus non-Stem cell treated groups under different force levels and to assess the bone formation around those mini implants. 15 New Zealand white adult male rabbits were randomly divided into three groups as; stem cell, stem cells+boron and control. 77 titanium customized orthodontic miniscrews (Osmed Dental, Istanbul, Turkey) with a length of 6 mm and a diameter of 1.4 mm were implanted in each proximal tibia and different force levels (0, 150, and 300 cN) were applied to each group. In a 21-day retention period, the implant stability was assessed by resonance frequency analysis (RFA) and Implant Stability Quotient (ISQ) values were measured by Osstell device (Integration Diagnostics AB, Göteborg, Sweden). At the end of the study MSIs were evaluated 3 dimensionally with micro Computed Tomography (μCT); (SkyScan 1172; Bruker Cooperation, Kontich, Belgium). Descriptive statistics were performed for the statistical analysis. Since the data were not normally distributed, non-parametric tests were used. All baseline values and sub-group differences were compared by Kruskal-Wallis test. The data revealed that primary stability of all miniscrews was similar in all groups under 0 cN ($p = 0.135$), 150 cN ($p = 0.779$), and 300 cN ($p = 0.498$) force levels at the start of the experimental procedure. At the end of the study no statistically significant changes were determined in ISQ values of any force groups, under 0 cN ($p = 0.057$), 150 cN ($p = 0.893$), and 300 cN ($p = 0.972$) force levels. μCT findings suggest that Bone Volume to Tissue Volume ($p = 0.053$), Bone Surface to Bone Volume ($p = 0.061$), Trabecular thickness ($p = 0.061$), Trabecular number ($p = 0.099$), Trabecular separation ($p = 0.159$), closed porosity ($p = 0.097$) and open porosity ($p = 0.098$) value changes were not statistically significant in 3 main groups. The null hypothesis was accepted that there is no significant difference between the orthodontic mini screw stability of Stem cell-treated versus non-Stem cell treated rabbits.



**Key Words: Stem Cells, Mini Screw Implants, Bone Formation, Mini Screw Stability, Boron**

## TURKISH ABSTRACT

**Mesgarzadeh, N. (2018). Adipoz türevi mezenkimal kök hücrelerin ortodontik mini vida implantlarının stabilitesi ve kemik oluşum üzerine etkileri. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Ortodonti Doktora Tezi, İstanbul.**

Bu randomize kontrollü deneysel hayvan çalışmasının amacı, Kök Hücre uygulanmış ortodontik minividaların stabilitelerini ve çevresindeki kemik oluşumunu değerlendirmektir. Bu sebeple 15 Yeni Zelanda beyaz yetişkin erkek tavşan rastgele üç gruba ayrılmıştır; kök hücre, kök hücre+bor ve kontrol. Her bir proksimal tibiaya 6 mm uzunluğunda ve 1.4 mm çapında 77 titanyum ortodontik (Osmed Dental, İstanbul, Türkiye) implantlar yerletirilmiş, ve her bir gruba farklı kuvvet düzeyleri (0, 150 ve 300 cN) uygulanmıştır. 21 günlük bir retansiyon periyodunda implant kabiliyeti rezonans frekansı analizi (RFA) ile değerlendirilmiş ve İmplant Stabilite Parçası (ISQ) değerleri Osstell cihazı (Entegrasyon Diagnostics AB, Göteborg, İsveç) ile ölçülmüştür. Çalışmanın sonunda minividalar Mikro Bilgisayarlı Tomografi (μCT) ile 3 boyutlu olarak değerlendirilmiştir; (SkyScan 1172; Bruker İşbirliği, Kontich, Belçika). İstatistiksel analiz için tanımlayıcı istatistikler yapılmış ve Veriler normal dağılmadığından, parametrik olmayan testler kullanılmuştur. Tüm başlangıç değerleri ve alt grup farklılıkları Kruskal-Wallis testi ile karşılaştırılmıştır. Veriler, deneysel işlemin başlangıcında 0 cN ($p = 0.135$), 150 cN ($p = 0.779$) ve 300 cN ($p = 0.498$) kuvvet seviyelerinin altındaki tüm gruplarda tüm mini vidaların primer stabilitesinin benzer olduğunu ortaya koymuştur. Çalışmanın sonunda, herhangi bir kuvvet grubunun ISQ değerlerinde, 0 cN ($p = 0.057$), 150 cN ($p = 0.893$) ve 300 cN ($p = 0.972$) kuvvet seviyelerinde istatistiksel olarak anlamlı bir değişiklik saptanmamıştır. μCT bulguları kemik hacminin doku hacmine oranı ($p = 0.053$), kemik yüzeyinin kemik hacmine oranı ($p = 0.061$), trabeküler kalınlık ($p = 0.061$), trabeküler sayı ($p = 0.099$), trabeküler ayırım ($p = 0.159$), kapalı porozite ($p = 0.097$) ve açık porozite ($p = 0.098$) değeri 3 ana grupta istatistiksel olarak anlamlı bulunmamıştır. Sıfır hipotezi, Kök Hücre uygulanmış ve Kök Hücre uygulanmamış tavşanlara karşı ortodontik mini vida stabilitesi arasında anlamlı bir fark olmadığı kabul edildi.

**Anahtar Kelimeler: Kök Hücreler, Mini Vida İmplantları, Kemik Formasyonu, Mini Vida Stabilitesi, Bor**

## 1. AIM and INTRODUCTION

Forces and moments are the core bases in orthodontics. The orthodontic forces are reciprocal; which means that they are in the same magnitude but opposing direction. For a successful treatment result, the clinician should manage any unwanted tooth movement. The term “anchorage” in orthodontics is the ability to resist an unwanted reactive tooth movement, this can be provided by other teeth, palate, head or neck or implants in bone (1).

Orthodontic mini screw implants (MSIs) gained its popularity due to the fact that it can facilitate maximum anchorage, and eliminate the use of extra oral appliances. In this scenario the treatment predictability is no longer relied on the patient compliance. Orthodontic MSIs became an efficient tool in orthodontics in recent years, but there is still a need to minimize the risks and to uncover the factors which leads to the failure of orthodontic MSIs (2).

In the literature the success rate of a MSIs has been reported from less than 50% to over 95%. The variable predictability of MSI stability limits their effectiveness as a treatment modality. When a MSI fails, not only it delays the treatment duration, but the patient may have discomfort and the treatment mechanics or plans may change.

MSI failures have been correlated with several factors from patient hygiene and soft or hard tissue characteristics of the MSI site, to the issues that could occur upon loading.

Primary stability which is mostly relied on the physical properties of an implant, is considered a mechanical aspect and is the core factor for implant success. Secondary stability will be achieved upon new bone formation and remodeling around an implant which already has a primary stability. Mobility has been reported as the most frequent cause of MSIs failure, and improvements in various MSIs design continues to enhance primary stability (3).

Accelerating osseointegration, healing and remodeling process is a proved method to increase the success rate of MSI. According to Ikeda et al., there is a challenge to maintain the success rate of orthodontic MSIs when heavy forces are going to be applied or when other anatomical or physiological factors are not ideal (4).

The use of various forms of biophosphonate derivatives and other pharmacological methods or use of Platelet-Released Growth Factor therapy or light-emitting diode (LED) photobiomodulation therapy (LPT), low-energy laser irradiation and low-intensity pulsed ultrasound exposure are the new techniques for enhancing the ability of bone regeneration around MSIs (5). These kind of novel biologic methods opens a new horizon at the next level of improving the stability of MSIs (6- 11).

A new method for accelerating bone formation is implementing bone regeneration techniques, specially stem cell therapies, which in dentistry has gained popular attention in the field of dental implantology. However, studies about intervening rehabilitation at the cellular level and the use of mesenchymal stem cell therapy on mini screw stability are not found in the reviewed literature. Thus, the aim of this experimental animal-based study was to evaluate the effect of adipose tissue-derived mesenchymal stem cells (ASCs) on the stability of immediately loaded miniscrews under different force levels. The use of boron in activation of stem cells was evaluated as well.

For this randomized controlled experimental animal study, the null hypothesis was set as; there is no significant difference between the orthodontic mini screw stability of Stem cell-treated; with or without Boron versus non-Stem cell treated rabbits under different force levels.

## 2. LITERATURE REVIEW

### 2.1. Search MeSH Terms

A computerized literature survey was conducted using different databases: MEDLINE database (www.ncbi.nlm.nih.gov), Scopus (www.scopus.com), and Web of Knowledge (apps.webofknowledge.com). A systematic search was conducted for conference abstracts published by the most important dental scientific societies up to December 2017.

A search of electronic databases including Ovid (MEDLINE), PubMed, and Cochrane Central for studies was performed limited to articles published in English. The literature search build used the combinations of keywords as follows:

Search 1. ((((((((((((((mini screw[Title]) OR mini screws[Title]) OR miniscrews[Title]) OR miniscrew[Title]) OR mini-screws[Title]) OR mini-screw[Title]) OR TADs[Title]) OR temporary anchorage devices[Title]) OR mini implant[Title]) OR mini implants[Title]) OR mini-implant[Title]) OR mini-implants[Title]) AND stability[Title/Abstract]) NOT case report[Title]) NOT overdentures[Title/Abstract]) NOT dentures[Title/Abstract]) NOT finite[Title]
Search 2. ((stem cells[Title]) OR stem cell [Title]) AND bone regeneration[Title]
Search 3. ((stem cells[Title]) OR stem cell [Title]) AND dental implant[Title]
Search 4. Stem[Title] AND boron[Title]

Exclusion criteria included:

1. Articles on standard dental implants, except the ones that used any kind of stem cell therapy

2. Case reports and case series

3. Articles not available in English language

The articles were first selected after reading their titles and abstracts. All of the articles that appeared to meet the inclusion criteria on the basis of the abstracts were read, and further selections were made. To encompass all of the above-mentioned terms for mini implants in orthodontics in one definition, mini screw implant (MSI) term were used in this research.

## 2.2. Mini Implants

Achieving anchorage control is one of the most important goals of clinical orthodontics (3). In earlier years; using teeth, extraoral or intraoral appliances as an anchorage caused the orthodontists to rely solely on the patient compliance.

**Dental implants**, when osseointegrated, helped the orthodontists to achieve maximum anchorage, but it is not a routine to use them comfortably for the purpose of reinforcing anchorage. Nowadays when they are already present in the mouth, they could serve as an Anchorage unit if needed.

**Mini-implants** are very similar to endosseous implants. They are conical and connection with orthodontic appliance is through the head of the implant that is positioned out of the mucosa. However, osseointegration in this kind of implants is compromised, because of its smooth surface.

**Mini-plates** are composed of a baseplate and fixation screws. They can be used either for orthodontic anchorage purposes or in maxillofacial surgeries.

**Mini-screws** are specially designed for orthodontics and mostly composed of titanium alloy. They are more tapered and smaller than endosseous mini implants, and their diameter does exceed from 2 mm (12).

Current MSIs, introduced by Kanomi, have become increasingly widespread in the recent years (13). MSIs are small in size, noninvasive, reliable and affordable temporary anchorage devices; their placement and removal of them could be accomplished easily in the orthodontist clinic. Their reported success rates in the literature range from about 50% to 95% (14, 15). MSIs usage reduces the side effects of an unwanted tooth movement by moving the teeth more controllably (16). Nowadays orthodontists are able to perform some orthodontic mechanics that were extremely difficult or impossible to do with conventional techniques. Mesialization of a molar in a patient with several missing teeth, distalization or intrusion of maxillary and mandibular teeth are facilitated with the use of MSIs (17). Schatzle et al. and Stanford et al. (18, 19), in two systematic reviews reported mean overall failure rates of 16.4% for orthodontic mini screw implants.

Factors affecting the success and failure rate of mini-implants have been divided into different categories. These categories are patient, mini-implant, orthodontic and

surgical factors. The factors that directly associates with the patient history before placement of MSIs can be listed as patient age, skeletal pattern and oral hygiene (20, 21).

**Age**

According to a meta-analysis including 50 articles published in European Journal of Orthodontics in 2014, the thickness and density of the cortical bone usually guarantees mechanical retention. In the older patients with adequate bone density, MSIs are retentive and fewer failures are expected. However, in adolescent patients the use of compliance-dependent appliances could be preferred to the use of MSIs in order to allow growth modification in these young patients (12).

**Skeletal Pattern**

Facial pattern is another factor that plays an important role in the success rate of orthodontics MSIs. Miyawaki et al.(23) pointed to this important factor in 2007. More recently, the cortical bone thickness of the alveolar process of patients with different Frankfort-mandibular plane angles (FMA) had been assessed with cone-beam computed tomography in patients, and it has been shown that patients with high FMA demonstrated lower success rates of mini implants, due to the reduced cortical bone thickness, which may affect primary stability of the MSIs (22- 24).

**Oral hygiene**

Failure rates increases with poor oral hygiene. Although it may seem obvious that poor oral hygiene causes MSIs failure, a controversy in literature exists in this regard (25). Sharma et al. (26) reported that poor oral hygiene and inflammation were associated to MSI failure. On the other hand, Park et al. (20) found that oral hygiene has no role and what is important is the local inflammation around MSIs.

In a study Uribe et al. (25) assessed the failure rates of mini-implants placed in the infrazygomatic region. They showed that this controversy is due to the fact that the type of mucosa adjacent to the MSIs is directly associated with the success of MSIs. Non-keratanized tissue can be a possible risk factor for MSIs failure. Non-keratanized gingiva can develop an inflammatory process when dental plaque exists, and this vulnerability compromises mini implant stability.

### 2.2.1. Risk and Complications of Orthodontic Mini Screw Implants

The lack of enough stability during orthodontic treatment is the most important factor that the clinician encounters during the treatment with MSIs. However, compromised stability is considered as one of many risks that could occur during orthodontic therapy with mini implants. Generally, the complications of orthodontic MSI may be categorized as follows;

#### 2.2.1.1. Complications During Insertion

**Trauma to the periodontal ligament or the dental root**

When placing a mini implant between the roots that are proximal to each other, there is a risk to impinge the dental root or the periodontal ligament. When the damage is limited to the outer root and does not invade dental pulp, the prognosis of the teeth does not change and the problem will resolve after 3 to 4 months. When the invasion is more severe, the tooth may loose its vitality or ankyloses may occur. In order to minimize the risks of root damage, proper radiographic imaging is crucial prior to any implant placement (27- 31).

**MSI slippage**

In some anatomical areas such as the zygomatic buttress, the retromolar pad, the buccal cortical shelf, and exostosis areas if present in the buccal maxilla, during the placement of MSIs, the implant may not fully engage with the cortical bone, and it may slip and harm some important anatomical tissues. For example; any uncontrolled slippage of mini implant in the retromolar pad, may cause an iatrogenic damage to the lingual and inferior alveolar branch nerves. Precise control in the angle of the placement, by engaging the implant initially in the bone and gradual reduction of angle and controlled force according to the bone density during the placement can reduce the risk of mini implant slippage (31, 32).

**Nerve involvement**

Slope of the palate in maxilla, the mandibular buccal alveolus, and the retromolar pad are the areas in which the clinician should place the mini implant with more care. When in palate, the implants should not be placed distal to the second molar, and the position of greater palatine foramen with the exiting nerve should be in mind during placement. In mandibular buccal alveolus, care should be taken to avoid to the mandibular canal and mental foramina specially in edentulous patients. In retromolar area, long implants may impinge the long buccal or lingual nerve (31).

**Air subcutaneous emphysema**

If the pilot hole is to be drilled prior to implant placement working on a loose alveolar tissue or zygomatic regions, the process of pilot hole preparation should be undertaken with low rotary hand peace. Bleeding and saliva should be controlled with suction or cotton. Using air water syringe and high speed hand piece may cause the air to penetrate and distort the skin or submucosa (31).

**Nasal and maxillary sinus perforation**

The zygomatic process, posterior or anterior maxilla are the regions which hold the risk for sinus perforation during MSI placement. In the edentulous areas, the risk of perforation should be minimized by changing the angulation of MSI. If the perforation happens, follow up for the development of sinusitis and mucocele is mandatory (33).

**MSI bending, fracture, and torsional stress**

Drilling a pilot hole when the insertion area has a dense bone, minimizes the torsional stress and reduces risk of MSI bending or fracture, or produce small cracks in the peri-implant bone, that affect MSI stability. De-rotation of the MSI one or two terns reduces the stress in the dense bone. At removal; the hand driver handle should be gently separated from its shaft in order to reduce any unnecessary stress to the MSI (31, 34).

#### 2.2.1.2. Complications During Loading

**Primary anchorage failure**

Primary anchorage is directly associated with the bone density. Bone density is categorized into 4 groups based on Hounsfield units (HU). This is accomplished when an x-ray unit using computer tomography scans and characterizes the bone density. D1 (>1250 HU) is dense cortical bone primarily found in the anterior mandible and the maxillary midpalatal area. D2 (850-1250 HU) is thick (2 mm), porous cortical bone with coarse trabeculae primarily found in the anterior maxilla and the posterior mandible. D3 (350–850 HU) is thin (1 mm), porous cortical bone with fine trabeculae primarily found in the posterior maxilla with some in the posterior mandible. D4 (150–350 HU) is fine trabecular bone primarily found in the posterior maxilla and the tuberosity region. Osseointegrated dental implants placed in D1 and D2 bone showed lower stresses at the implant-bone interface. D1-D3 bone are optimal for self-drilling miniscrews. Placement of miniscrews in D1 and D2 bone might provide greater anchorage under orthodontic loading, unlike the D4 areas which has a high failure of MSI (31, 35).

**MSI design and surgical techniques**

When the mini implant is going to be placed in the position with unfavorable conditions, various modification in implant may assist to overcome the imperfect situation. One of these modification is to change the surface characteristics of an implant in order to increase the rate of osseointegration between the titanium dioxide layer of an implant and surrounding bony tissue. Other studies focus on macroscopic design elements such as thread and taper design. Different lengths and diameters as well as conical or cylindric shapes were tested for their influence on primary stability (36, 37).
Even though miniscrews are sometimes believed to achieve their retention purely mechanically, Cope et al.'s study (38) pointing to the paradigm shift in temporary anchorage devices in orthodontics insisted that the idea of osseointegration should not be dismissed entirely. Partial osseointegration has been shown in both in-vitro and in-vivo studies (39 - 57). Vande Vannet et al. (39) performed a histomorphometric study in dogs, in which the extent of osseointegration was investigated. They assessed the appearance of the bone microscopically that was in close proximity to the screw and found that the

overall mean osseointegration of all screws was calculated at 74.48 per cent (±15.63 per cent).

The other factor which has been used to categorize orthodontic mini-implants is self-tapping and self-drilling, depending on the position of their thread. Self-tapping mini-implants have a tapered design and a blunt tip, and their threads are guided around a cylindric core spirally. On the contrary, self-drilling mini-implants have a sharper conical tip and their threads are machined from the tip along an axis of rotation to the neck. Su et al. (58) in an animal study assessing the insertion torque and displacement under lateral loading, compared self-tapping and self-drilling orthodontic mini-implants and demonstrated that self-tapping designs had lower insertion torques and less implant-bone contact than self-drilling designs.

In addition to these, torque, depth and the angle of insertions are all considered important factors in MSI stability. When the factors combine with experience of the practitioner and highly controlled procedure implementation; it can prevent any possible failure (20, 59, 60).

**Loading Force**

According to Serra et al. (58), the load per se does not cause the loss of stability until an overload limit. Several studies assessed different loads tolerated with various implant designs. The overloading limit is influenced by the implant design; the first screw threads of mini-implants have stress concentrations after a lateral or an oblique load, causing marginal bone loss (62, 63). There are no reports on the maximum force tolerated by a mini-implant. However, most studies used a force of 1.5 to 3 N to test mini-implants stability (64- 66).

**Miniscrew migration**

Miyawaki et al. (23), stated that peri-implant soft-tissue type, health, and thickness can affect primary stability of the MSI. Orthodontic MSIs usually remain clinically stable but this does not mean that they are absolutely stationary under orthodontic loading. The scenario is different than in an endosseous dental implant that osseointegrates with the bone. For orthodontic MSIs primary stability is achieved through mechanical retention, but still the mini implant can be displaced within the bone. Liou et al. (67) comparing cephalometric radiographs of 16 patients, reported that

orthodontic MSIs in zygomatic buttress loaded with 400 g of force for 9 months extruded and tipped –1.0 to 1.5 mm in 7 of 16 patients. To leave room for potential migration, leaving a 2-mm safety clearance between the MSI and any anatomical structures have been suggested.

**Soft-tissue coverage of the MSI, inflammation, and peri-implantitis**

Coverage of MSI with soft tissue might compromise mini screw stability. The MSI placed in the mandible is more prone to soft tissue coverage, this overgrowth can concern the patient, who might think that the mini implant has fallen out. Placing a healing cap, wax or elastic can minimize this phenomenon. The use of chlorhexidine mouthwash with its antibacterial properties slows down the epithelialization process. Also, when the soft tissue twists around the mini implants shaft during placement, the risk of inflammation increases (68).

### 2.3.2. Methods of Measuring Stability for Dental Implants

Severe clinical mobility of a mini implant is considered as a failure of the mini implant. In this situation mini implant can no longer act as a stationary anchor. The clinician needs to remove or replace the mini implant to continue as the treatment protocol (21, 69- 71). Meursinge Reynders et al. (60) in their systematic review concluded that; “success is a subjective qualitative recording of stability and should not be considered as a reliable measure for testing”. On the other hand, recordings with objective measuring devices should become the gold standard for testing the associations between the stability of orthodontic mini-implants and independent variables. Torque measurements, the periotest device, and resonance frequency analysis (RFA) are used extensively in-vivo and in-vitro stability assessment studies (72).

#### 2.3.2.1. Torque Measurements

Removal torque of orthodontic mini implants has been investigated by some researchers to evaluate the implant- bone interface. The force required for removing a mini- implant is related to the torsion resistance of the implant-bone interface and thus can be used to evaluate anchorage capability indirectly. Motoyoshi et al., in a study

assessing the factors that affects the long-term stability of orthodontic mini implants stated that; to improve the initial stability and anchorage capability of a mini-implant, it is necessary to identify factors that affect placement and removal torques. They defined that the removal torque was not significantly related to placement torque and the most widely approach in the literature is to measure maximum insertion torque (MIT) during insertion of a mini implant. For this purpose, dedicated surgical motors in clinical practice and meticulous torque sensors in the laboratories are used (71- 75). Suzuki et al. (74), in a study of human cadavers inserted two hundred self-drilling mini screw implants. They recorded maximum insertion torque value during implantation procedures. Meanwhile assessment of primary stability was carried out immediately after implantation using the RFA method, and implant stability quotient (ISQ) values were recorded. They have concluded that implant stability using resonance frequency analysis are highly correlated with maximum insertion torque.

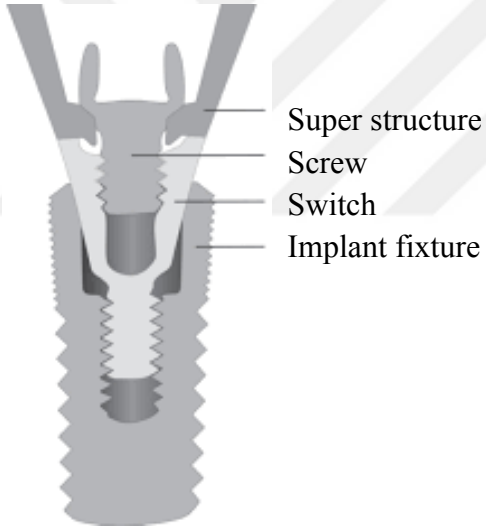


**Figure 2.1:** Schematic view of a torque measurement device (75)

#### 2.2.2.2. Periotest

Another principle of measuring stability, which is used by the Periotest device (Figure 2.2), is to capture the damping characteristics of an implant (76). This method, first developed to measure tooth mobility, and then was introduced to implant dentistry by Bragger et al. by percussing the implant head with a small pestle that will rebound at

a specific speed depending on implant stability. During contact, a piezoelectric crystal inside the head of the pestle is deformed, thus, creating an electric impulse that reveals the duration of contact. This time-based information is converted to stability expressed as Periotest values (PTV), which range from -8 to +50 (77).


**Figure 2.2:** The Periotest Device

### 2.2.2.3. Resonance Frequency Analysis (RFA)

A third method widely used is Resonance Frequency Analysis (RFA). In this case, a SmartPeg with a permanent magnet is tightened into the implant. A hand piece emits electromagnetic impulses of 5–15 kHz against the peg to record the resonance frequencies, which are converted to stability expressed as ‘‘implant stability quotient’’ (ISQ) values ranging from 0–100 (77). RFA, a well-established technique for evaluating the longitudinal stability of endosseous implants, is currently considered as a non-invasive way to monitor implant stability. Most recently, the use of RFA to measure MSI stability has been validated and applied clinically in humans. ISQ stands for Implant Stability Quotient and drives from Herz since the technique it uses is RFA. The scale in Herz is non-intuitive and the hard to communicate so it was translated to scale from 1-

100 ISQ more than 20 years ago. Today we know that this scale correlates to micro mobility (Figure 2.3).


**Figure 2.3:** The Osstell Device (Integration Diagnostics AB, Göteborg, Sweden)

The resonance frequency depends on the dimensions and material of the SmartPeg, and the boundary conditions between SmartPeg and the implant. when the connection between SmartPeg and the implant has been accomplished successfully, boundary conditions shows the amount of implant fixation in bone; which is regarded as "implants stability". Thus, if the dimensions and materials of the SmartPeg is set, frequency of the SmartPeg will depend on only the implant stability. If the stability of implant is measured above the bone level, it will be lower than measured at bone level. This is because the part of the implant above bone will act as a prolonged part of the SmartPeg, and the resonant frequency (and ISQ) will therefore be lower. If there is a marginal bone loss around the implant, it will show as a lower ISQ. Osstell measures the lateral stability of the implant and thereby indirectly the degree of osseointegration. The SmartPeg with its magnet on top, works like a small tuning fork. The magnet is emitted with magnetic pulses from the probe which makes the SmartPeg vibrate. Due to the stiffness in the interface between the implant surface and the bone, the SmartPeg will vibrate accordingly (Figure 2.4). The denser bone the higher stability, the higher frequency and the higher ISQ value (78- 80).

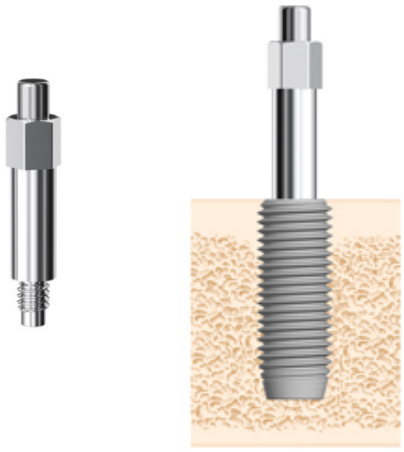

**Figure 2.4:** The Smartpeg

**Stability and Osseointegration**

High percentage of bone contact in soft bone may be present at the same time that an implant shows low stability. Likewise, in a dense bone, less contact percentage exists but the higher instability is also possible. This is explained by stiffness in the bone itself. In soft bone a low ISQ at placement may be recorded and increased stability value due to osseointegration may be observed. If you have a high initial mechanical stability already at placement, osseointegration will not add stability in a significant way, because the implant was already stable. For the best results, the probe should be held perpendicular to the bone line, and in-line with the bone for the other measurement (Figure 2.5) (79).

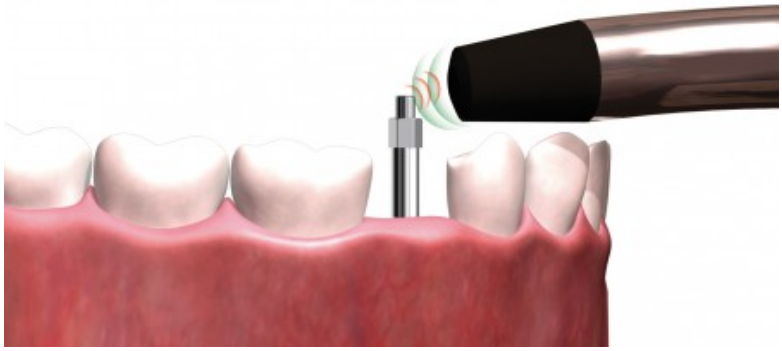

**Figure 2.5:** Probe should be held perpendicular to the bone line (79)

### 2.2.3. Methods of Enhancing Mini Implant Stability

#### Modifying Surface Topography

Methods for modifying the surface topography are sandblasting, acid-etching or anodic oxidation of the miniscrews or coating the miniscrew with titanium and nitrogen by plasma ion implantation, or photofunctionalization. These methods can probably increase the bioactivity of titanium-alloy miniscrews and improves the anchoring capability of orthodontic miniscrews (81). The microgroove on the implant surface is known to play a role in proliferation and migration of fibroblasts, formation of thick connective tissue, the adaptation effect of connective tissue, and, finally, maintenance of soft tissue around the implant. In 2008 Kim et al. (82) searched the effects of microgrooves on the success rate of MSIs in dogs histologically, and they found that microgroove on the implant surface could exert some positive effects on soft tissue adaptation and bone healing. These methods are examples of using some modification in the designs of the mini implants that have influence on the cell level

#### Modifying the Macroscopic Design

Miyawaki et al. (17) showed that novel spike-like auxiliary skeletal anchorage device may increase miniscrew stability, allow a shortened mini screw, and enable 3-dimensional absolute anchorage. A larger-diameter miniscrew showed a significant increase in insertion torque in a study of Lim et al. (83). In addition, a tapered miniscrew displayed maximum insertion torque compared with a cylindrical miniscrew (84). However, the length of the miniscrews had no effect on the survival rate of mini screw as stated by Miyamoto et al. (85).

In 2012 a systematic review assessing insertion torque and success of orthodontic mini-implants, suggested that there is no evidence to recommend specific maximum insertion torque levels that leads to a higher success rates of orthodontic mini-implants. In their study, Meursinge Reynders et al. (60) found that an association between specific maximum insertion torque values and success of orthodontic mini-implants was analyzed only in nonrandomized studies of low quality.

**Alternating the Insertion Region**

Alternative insertion regions other than alveolar bone were investigated in order to enhance the stability of MSIs; examples of these regions are the maxillary tuberosity, the zygomatic buttress, or the retromolar region of the mandible. The anterior palate was found to be a favorable insertion site for orthodontic mini-implants (86).

Amount of bone tissue surrounding the screw is another important factor which was defined by Poggio et al. (29) as safe zone and assessed by Asscherickx et al. (87) in a study of the root repair after injury from miniscrew. They suggested that the minimum amount of bone tissue surrounding the screw to guarantee its stability should be 1 mm.

Miyawaki et al. (23) suggested a higher success rate for miniscrews inserted in the posterior part of the mandible than in the maxilla due to the greater thickness of the bone cortex. On the contrary; Cheng et al. (16) and Kuroda et al. (88), reported a significantly higher success rate for miniscrews inserted in the maxilla than for those in the mandible. In all the cases studied, miniscrews were not applied in the anterior part of the dental arch. Also, Berens et al. (89) found that success rates were always higher for miniscrews inserted in the maxilla. These controversial results may also be influenced by other factors, such as the greater amount of keratinized tissue, the less demanding surgical procedure, and the greater vascularization of the upper jaw (90- 92).

The fact that should be considered in all of the insertion regions is the presence of the attached gingiva. Generally, a better prognosis for miniscrews located in the attached gingiva is stated in previous clinical retrospective studies (93).

**Alternating the Insertion Protocol**

Wilmes and Drescher (94) showed that insertion depths result in higher insertion torques and thus results in a favorable primary stability. Larger predrilling diameters result in lower insertion torques.

In clinical circumstance it has been shown that placing screws not perpendicular to the bone surface, but at an obtuse angle, lowered the risk of root damage and increased the screw's contact with cortical bone (20). Liou et al. (95) analyzed the infrazygomatic crest in computed tomography scans and concluded that miniscrews should be placed at a steeper angle in this area. Araghbidikashani et al. (96)in an in-vitro study, assessing the impact of insertion angle on primary stability of miniscrews added that the direction of

the applied force had a significant impact on the primary stability of miniscrews at various insertion angles.

**Timing in Load Application**

A correlation between the time of force application and success rate is not always found and in comparison of some studies on consecutive patients, it is possible to find discordant results (23).

**Effects of Pilot Holes**

In 2014 Carnet et al. (11) assessed the effects of pilot holes on MSI stability to determine whether the effects can be attributed to the quality or the quantity of bone surrounding the MSI, longitudinally. Randomized split-mouth design in 6 dogs revealed that MSIs placed with pilot holes show greater primary stability, but greater decreases in stability over time, due to primarily having less trabecular bone surrounding them. Placement of MSIs without pilot holes became popular with the advent of self-drilling MSIs. Pilot holes came back in favor after 2006 because of concerns about screw breakage during placement and high insertion torque that may be linked to premature MSI failure (3, 97, 98).

## 2.3. Stem Cells

Stem cell technology for regenerative therapies is already available in dentistry, as mesenchymal stem/stromal cells (MSCs) already have been introduced in dentistry for alveolar bone augmentation. However, the translational utility of stem-cell-based technologies is still uncertain. The effectiveness of such approaches when compared with already- established regenerative techniques has not yet been properly evaluated, especially when considering their high cost and labor required. The concept of regenerative dentistry has been developed not only for oro-maxillofacial reconstruction of tissues loss due to trauma or cancer but also it has been applied in the fields of periodontology and implantology. In periodontal disease, which is a common cause of alveolar bone and tooth loss that limits the ability of dental implants to restore the periodontal anatomy or missing teeth, regenerative therapies aids the clinician to reconstruct the damaged site (99).

In orthodontics the idea of implementing stem cell and regenerative therapies is not so common and to-date there are two studies that used stem cell therapy in published orthodontics journals.

Ekizer et al. (100) in 2015 in a randomized control animal study transplanted bone marrow–derived mesenchymal stem cells (MSCs) into the interpremaxillary suture after rapid maxillary expansion with the aim of increasing new bone formation in the suture. Histomorphometric analysis revealed that a single local injection of MSCs into the midpalatal suture of rats increased the new bone formation in the suture by increasing the number of osteoblasts and new vessel formation, compared with controls.

Amuk et al. (101) in 2017, evaluated and compared therapeutic effects of mesenchymal stem cell (MSCs) and osteoprotegerin gene transfer applications on inhibition and/or repair of orthodontically induced inflammatory root resorption in rats. Transferred MSCs showed marked uorescence in PDL.The results revealed that number of osteoclastic cells, resorption lacunae, resorption area ratio, RANKL, and Cox-2 were reduced after single MSC injections significantly and subsequently as a result, inhibition and/or repair effect in orthodontically induced inflammatory root resorption by MSCs were confirmed.

### 2.3.1. Definition of Stem Cells

Stem cells are unspecialized cells with the ability to proliferate and differentiate to multiple cell types when stimulated by both internal and external signals. Adult (somatic) stem cells that exhibit this plasticity are called pluripotent cells and can be found in bone marrow in the form of hematopoietic, endothelial, and mesenchymal (stromal) stem cells (MSCs). Other sources of MSCs in adult patients have been also identified such as adipose tissues (ASCs), lung, and teeth (perivascular niche of dental pulp and periodontal ligament) (102) (Figure 2.6) (102).

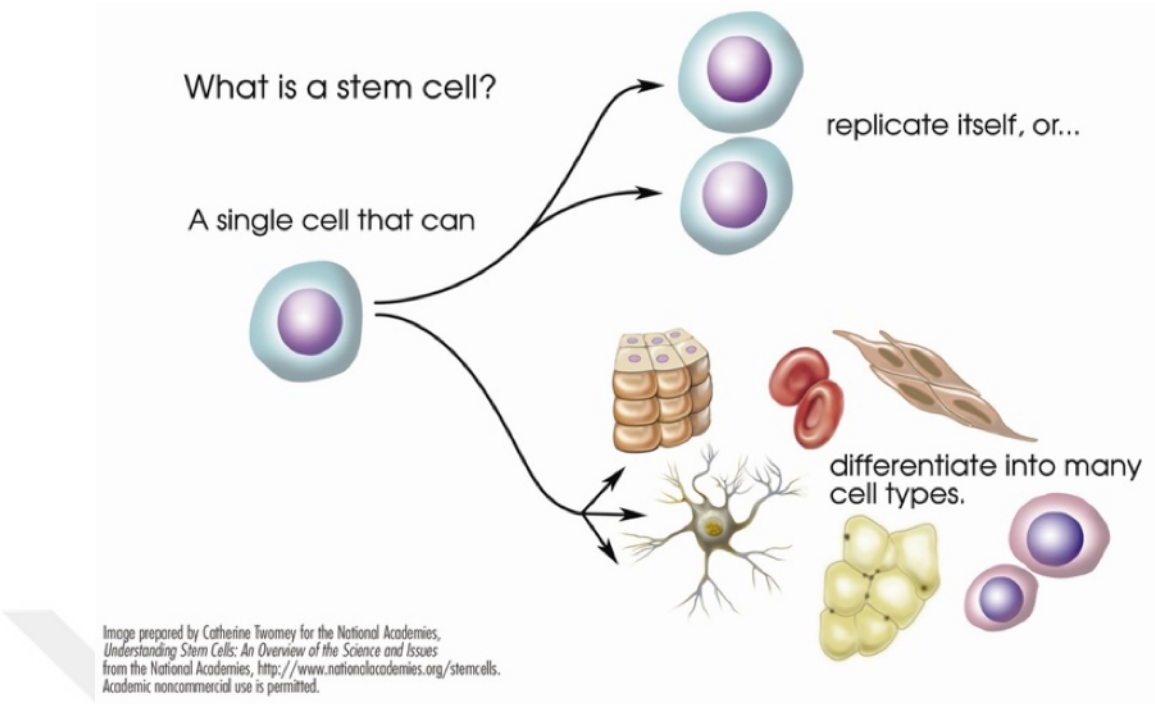


**Figure 2.6**: Stem Cell definition (102)

Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC: "Firstly, MSC must be plastic-adherent when maintained in standard culture conditions. Secondly, MSC must express CD105, CD73, and CD90 and lack expression of CD45, CD34, CD14 or CD11b, CD79a, or CD19 and HLA-DR surface molecules. Thirdly, MSC must differentiate to osteoblasts, adipocytes, and chondroblasts in vitro". In this way, MSCs can produce bone, cartilage, fat, or fibrous connective tissue depending on their differentiation process (102- 104).

### 2.3.2. Bone Biology

#### Bone Composition

The hierarchical structure of bone, extending from the macro-scale to the subnano-scale, is irregular and anisotropic. At the macroscopic level, compact outer cortical bone surrounds porous inner trabecular bone. Microscopically, cortical bone is composed of repeating osteon units containing collagen fibers and calcium phosphate crystals, whereas cancellous bone is an interconnecting framework of trabeculae with a

surrounding marrow space. A single osteon unit; (Figure 2.7) (105) consists of concentric layers of collagen fibers, called lamellae, running perpendicular to a central canal containing blood vessels and nerves.

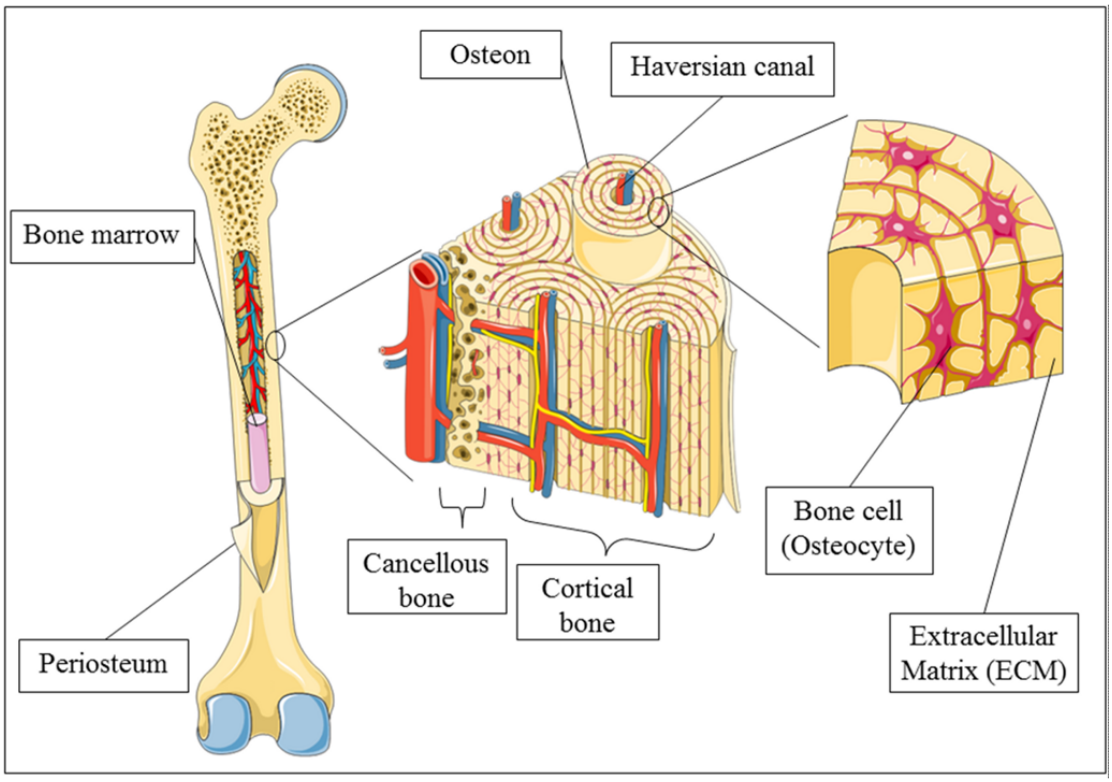


**Figure 2.7**: Schematic view of bone composition (105)

Bone is a mineralized dense connective tissue that consists mainly of a mineral component and an organic matrix. The organic matrix is composed primarily of type I collagen with less type III collagen. Type I collagen is a 300 nm long triple-helical structure that arranges into fibrils that form collagen fibers. Hydroxyapatite $Ca_3(PO4)_2(OH)_2$ nanocrystals are embedded between individual collagen molecules and serve to substantially increase the rigidity of bone. Together with non-collagenous proteins such as fibronectin, osteocalcin, osteopontin, and osteonectin, collagen forms a scaffold for mineral deposition, and proteoglycans such as decorin and biglycan regulate collagen fibril assembly and diameter. Type 1 collagen is evenly distributed throughout the skeleton, while non- collagenous proteins and proteoglycans are found in an irregular distribution pattern (105).

**Bone Development**

Cartilage, bone, and bone marrow stroma; like individual bones, arise at specific time points during development and from multiple embryonic lineages. Cranial neural crest gives rise to facial bones, while the remainder of the skeleton is derived from paraxial and lateral plate mesoderm. Furthermore, the development of the skeleton and its cellular hierarchy proceeds in a manner that is similar to – and tightly coordinated with the hematopoietic system. Skeletogenesis occurs through either intramembranous or endochondral ossification. Endochondral bone formation occurs through a cartilage intermediate that is replaced by bone and marrow. Mesenchymal condensations contain cells which transits to cartilage centrally and mature further into hypertrophic cartilage, later to be replaced by bone and marrow. The outer envelope of mesenchymal cells later give rise to primitive perichondrium of loose connective tissue and articular soft tissues. The perichondrium then gives rise to both chondrocytes and osteogenic cells. As the ossification centers and growth plates are later established, peripheral and hypertrophic chondrocytes contribute to osteogenic cell types. In membranous bones, chondrogenesis is typically aborted after transient expression of type II collagen as mesenchymal condensations undergo direct ossification (104).

**Bone healing**

Bone repair after fracture is a special process where sequential cellular and molecular events take place to generate new bone, rather than a fibrous scar like other connective tissues. The precise series of ordered events required to produce new bone are modulated by systemic and local factors, and disruption of these orderly events may cause healing problems. Thus, a clear understanding of the sequence of events and their regulation is needed to decide when and how an intervention is required to promote healing and to avoid complications. General pattern of indirect fracture healing, based on endochondral ossification, has included the chronological phases of haematoma, inflammation, angiogenesis, chondrogenesis to osteogenesis and finally bone remodelling. Direct healing based on membranous ossification, with no periosteal reaction or visible callus formation, is seldom seen. The well-established characteristics of the above mentioned phases require different processes of cell migration and differentiation, extracellular matrix formation and organization towards calcification, as

well as both local and systemic modulation. Apart from the classical histological phases of fracture healing, much remains to be understood about the regulation of these processes both at the molecular and the cellular level (107), (Figure 2.8) (108).

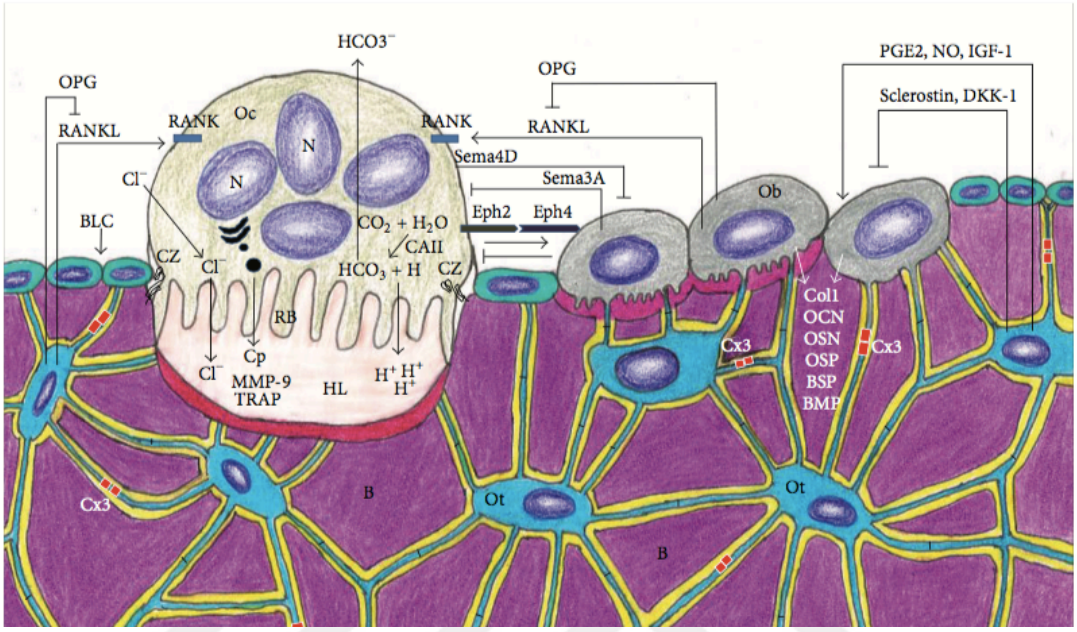


**Figure 2.8**: Schematic summary of bone tissue showing bone cells and the relationships among them and with bone matrix (108).

**Bone regeneration**

Bone regeneration is a dynamic process that balances the breaking down of old bone, the generation of new bone, and the infiltration of these areas with blood vessels. Two broad categories of cell populations available as sources of bone regeneration include osteoblasts and multipotent cells. Osteoblasts are cells committed along a bone lineage, possessing a limited number of divisions, and readily form mineralized matrix. Multipotent stem cells have the ability for prolonged division while maintaining the capacity to differentiate along multiple lineages with proper biological cues. Resident stem cells can be found in many adult tissues, and are active in endogenous mechanisms of repair and regeneration (109).

### 2.3.3. Adipose-Derived Stem Cells (ASC)

Currently all of the available options for skeletal tissue regeneration fall short of the ideal reconstructive methods. Autogenous grafts or alloplastic materials have inherent disadvantages such as unnatural texturing, inflammation, extrusion, resorption, and even rejection. They have limited availability, substantive morbidity, and may demonstrate poor viability. Thus, it remains a pressing need for a suitable alternative therapy for bone tissue repair. ASCs offer several advantages over other multipotent cells (such as bone marrow mesenchymal cells - BMSCs) for tissue engineering purposes. They are available in large numbers, are easily accessible, and attach and proliferate rapidly in culture. They have been described as mesenchymal stromal cells, with a proven ability to differentiate along osteogenic, adipogenic, chondrogenic and myogenic cell types, among others. Moreover, human ASCs show robust mineralization within 9 to 12 days of in-vitro differentiation. Numerous studies have attempted to utilize human ASCs for the regeneration of skeletal defects. It has been reported that allogenic mesenchymal stromal/ stem cells (MSCs) either derived from bone marrow or from circulative MSCs could be isolated and cultured in advance to achieve suitable implantation in clinical applications. However, the higher number of ASCs that can be isolated in one single step allows a more straightforward application of these cells particularly when time is of critical importance (110).

#### ASCs in Bone Regeneration

ASCs are able to directly differentiate into mature osteoblasts; moreover, such cells can produce chemokines that are useful for facilitating the homing of endogenous stem cells to the site of bone defect (111).

Surprisingly ASCs have the capability to release plasma membrane-derived vesicles (MVs) into the microenvironment: These vesicles are known to act as important mediators in cell-to-cell communication. MVs secreted by ASCs might deliver several types of molecules, such as growth factors, cytokines, RNAs, and microRNAs, even to distant locations throughout the body, and perform their biological activities on a number of target cells. ASCs can induce strong biological effects on target cells, such as promoting cell proliferation and differentiation, as well as the activation of regenerative and reparative processes. Since MVs are able to deliver osteogenic growth factors, such

as bone morphogenetic protein 2 (BMP-2), which may favor local bone tissue regeneration (112. 113), (figure 2.9) (114).

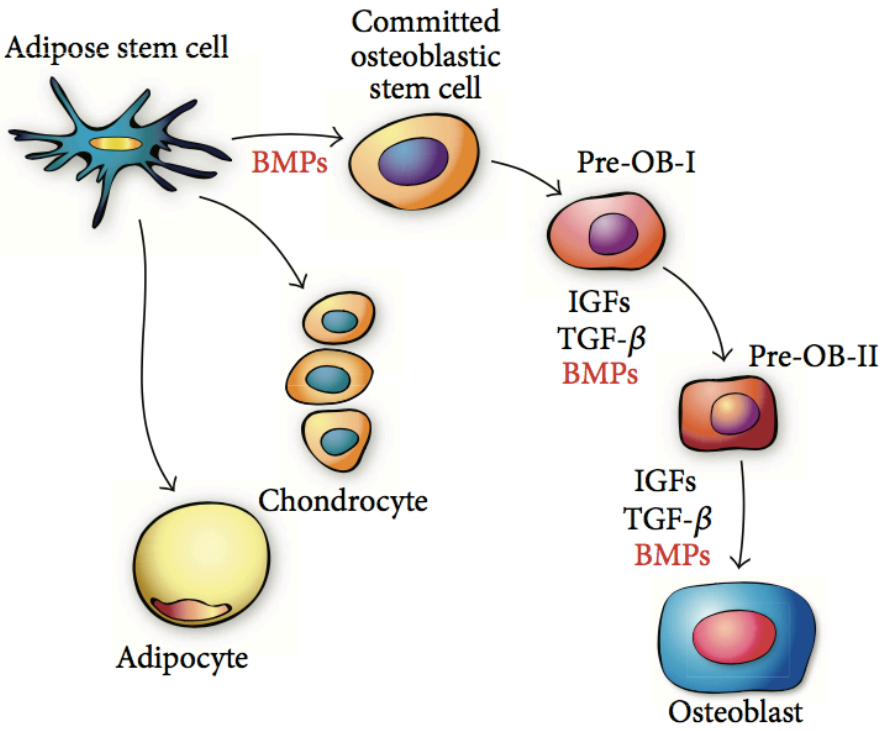


**Figure 2.9**: Schematic representation of BMP's effect on the differentiation of adipose stem cells into osteoblast (114).

**ASCs advantages**

In the scientific literature, ASCs have successfully been combined with biomaterials, such as β-tricalcium phosphate (β-TCP), bioactive glass, and platelet-rich plasma (PRP), to improve bone regeneration in animal models. Such studies on animals, together with several case reports on humans, provided interesting results in the field of bone regeneration. These results describe a new approach for bone tissue regeneration science, based on ASCs/scaffold constructs; however, a deep analysis of the studies reported in the literature would be useful to understand the real limits and perspectives of ASCs in bone replacement, from a clinical and molecular point of view (111, 115).

**ASC possible disadvantages**

Although ASCs have been indicated as a promising tool for the treatment of severe bone defects, it has been shown that donor age negatively affects the osteogenic commitment of ASCs, emphasizing a remarkable limitation in their therapeutic potential. ASCs derived from young donors showed a higher expression of osteogenic markers, such as osteopontin (OPN), osteocalcin (OCL), and BMP-2, and a higher content of mineral calcium deposits with respect to elderly patients. Thus, when considering using ASCs in bone reconstruction of the craniomaxillofacial region, it is also important to expect the notable influence of donor age on the proliferation and on the osteogenic differentiation of ASCs (114- 116).

**Molecular Characteristics of ASCs**

Bone healing is a complex process involving several molecular, biochemical, and cellular mechanisms: Among them, angiogenesis plays a particular role in the bone regeneration process. The formation of new blood vessels inside bone defects ensures the critical task of providing those sites with oxygen, nutrients, and growth factors, which facilitate bone formation. In this context, ASCs, when compared to other mesenchymal stem cells (MSCs), show a specific ability to secrete growth-factors, inducing angiogenesis, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and hepatocyte growth factor, indicating that ASCs are particularly suitable for bone tissue engineering. When compared to bone marrow stromal cells (BMSCs), ASCs show higher proangiogenic activities, mediated by the matrix metalloproteinases MMP-3 and MMP-9. Finally, ASCs secrete VEGF that are able to promote new blood vessel formation, and are also able to recruit hematopoietic stem cells. Such molecular characteristics make ASCs one of the most suitable MSCs targeting bone tissue engineering. ASCs also show the ability to secrete bone morphogenetic protein 2 (BMP-2), which plays an essential role in bone regeneration, as well as fibroblast growth factor-2 (FGF-2), keratinocyte growth factor, and insulin-like growth factor-1 (IGF-1), which are involved in the wound healing process (111).

Although several studies have reported a better commitment of BMSCs when compared to ASCs with respect to osteogenic differentiation, the mRNA levels of BMP-2, collagen type I, and osteonectin (ON) were expressed in higher amounts by undifferentiated ASCs than by BMSCs. In addition, it has been found that ASCs express

and secrete various growth factors and cytokines, such as receptor activator of nuclear factor κ-B ligand (RANKL), macrophage colony-stimulating factor, BMP-4, and extracellular matrix proteins, including fibronectin (FN) and type I collagen, which are involved in bone remodeling (111).

Finally, it is important to underline that ASCs can survive in low-oxygen environments, making them optimal for cell therapies where oxygen supplied by the vascular network may be limited, such as in implant surgery procedures. In fact, under hypoxic conditions, ASCs are able to increase VEGF secretion, stimulating the proper angiogenesis needed for bone formation (118).

Although ASCs have been extensively used in bone reconstruction, the molecular mechanisms that regulate the osteogenic commitment of these cells is still the subject of current researches (111).

### 2.3.4. Safety in Stem Cell Therapies

All cellular products must be in compliance with guidelines with respect to medicinal products and investigational medicinal products for human use. The European Union (EU) regulation on advanced therapy medicinal products was adopted by all European Member States on December 30, 2008 and the United States Food and Drug Administration (FDA) recently also proposed regulations on human cells, tissues and cellular and tissue-based products. The main scope of these regulations is to establish clear classification criteria for many new cell-based medicinal products. For the EU, it makes reference to the 2004/23/EC directive on donation, procurement and testing of human cells and tissues and also with directive 2002/98/EC on human blood and blood components. Key elements for cellular-based products include identity, purity, sterility, stability, safety and efficacy are recommended. In all, these new regulations impose strict criteria for the production and the environment used for the production of cell-based products to be used in clinical trials and treatments (119- 123).

#### Scaffolds

Engineering new bone tissue to repair and regenerate bone at bone defect sites represents one of the most challenging fields in regenerative medicine. Success of the procedure is determined by use of a suitable scaffold, a structural support for

osteoprogenitor cells, and osteoinductive factors necessary to regenerate neo-bone at the site of bone defect (124).

In general, bone defects are irregularly shaped. Therefore, the scaffold should allow for complicated and irregular bone geometry. A modern scaffold consists of three-dimensional (3D) interconnected pores that allow for uniform penetration of nutrients and removal of metabolic waste in-vivo to assist the development of new bone tissue. In addition, the scaffold must also be biodegradable to keep pace with the formation of the new bone tissue. Therefore, an ideal scaffold for bone tissue engineering should possess bone-like geometry and a 3D porous structure for bone defects, as well as be biodegradable to match the formation of a neo-bone tissue. Several scaffold fabrication techniques can be utilized to meet the requirements of the target bone defect. Recent research efforts have focused on the fabrication of a scaffold with complicated and interconnected pore structure, including several computer-designed scaffold fabrication techniques; for example, solid freeform fabrication. These pioneering techniques can be used to fabricate a customized and solvent-free scaffold with tailed bone geometry and interconnected pore structures for repair of bone defects (124).

### 2.3.5. Stem Cells and Dental Implants

Recent decade, the use of stem cell treatment in implantology has shown an out burst. Most of the researches used an animal model to test different treatment modalities, and the data is accumulating to facilitate the use various bone regeneration protocols more frequently in a clinical or surgical settings.

Zheng et al. (125) in a study of bone regeneration of blood-derived stem cells within dental implants suggested that; these kind of stem cells have the capacity to be considered as a promising source for bone regeneration in dental implant surgery.

Several animal studies were undertaken to evaluate the use of tissue-engineered bone as grafting material for alveolar augmentation when with immediate implant placement in canine models. The results suggested that tissue-engineered bone has the necessary qualifications to enhance of bone regeneration around dental implants (126-128).

Jhin et al. (129) delivered bone morphogenetic protein-2 gene to the bone marrow stem cells in rabbit, for maxillary sinus augmentation while placing an implant. These

results suggest that using BMSCs may result in earlier and increased bone formation in the maxillary sinus. This finding may offer more stable bone support to implants and reduce healing times. However, they stated that this kind of therapy has some limitations, the most important disadvantage is that the enhancing effect of BMP-2 will subside by time in later stages of bone healing around the implants.

Implementing a new method, Zheng et al. (130) used suspended cells which usually are discarded during the maintenance of bone marrow-derived mesenchymal stem cells (BMMSCs) for bone regeneration around the dental implants. Following the characterization of suspended BMMSCs from rabbit bone marrow by bioengineering, they applied the suspended BMMSCs to double-canaled dental implants in the rabbits. Their findings suggested that suspended BMMSCs, aided in the bone regeneration process around and in to the canals of the implants in the study groups.

Nagahara et al. (131) introduced of a mixture of β-tricalcium phosphate into a complex of bone marrow mesenchymal stem cells and type I collagen and assessed the volume of alveolar bone. Their findings suggest that β-TCP is considered a suitable scaffold for BMMSCs transplantation and helps augment alveolar bone without impairing regeneration of cementum.

In another approach; Yun et al. (132) in an animal study stated that Bone marrow-derived mesenchymal stem cells (BMMSCs) and platelet-rich plasma (PRP) may provide additional therapeutic effects on bone regeneration and improve osseointegration in bone defects around dental implants (133).

### 2.4.6. Boron in Stem Cell Therapies

In Botany, Boron is considered a fundamental element for plants, in the contrary in medicine the boron has been less understood and discussed. Studies had been revealed that the boron engages with in various aspects of growth in rats in embryo. Boron insufficiencies affects hard tissue health and growth. Taşlı et al. (134) assessed the odontogenic and osteogenic differentiation of human tooth germ stem cells in vitro using different concentrations of sodium pentaborate pentahydrate (NaB**).** Their study offers considerable promise for the development of new scaffold systems combined with NaB in both functional bone and tooth tissue engineering. Several studies associated the amount of boron with the level of bone formation and mineralization (135 - 141).

Demirer et al. (135) evaluated the histopathologic and morphometric effects of systemic boric acid in a rat periodontitis model and could prove the involvement of this element in periodontal health. Hakki et al. (136) in 2013 showed that boron has beneficial effects on bone strength and mineral composition in rabbits fed a high energy diet. More recently in 2016 Shaui et al. (137) demonstrated that boron nitride nanotubes reinforce tricalcium phosphate scaffolds and promote the osteogenic differentiation of mesenchymal stem cells. Uysal et al. (141) evaluated the effects of dietary boron on bone regeneration in rabbits in response to expansion of the midpalatal suture during different retention periods and concluded that Boron has a positive effect on the early phase of bone regeneration of the midpalatal suture in response to expansion and may be beneficial in routine maxillary expansion procedures.

## 2.5. Micro-Computed Tomography (μCT)

Clinical CT scanners typically produce images composed of 1 $mm^3$ volume elements (voxels). X-ray microcomputed tomography (Micro-CT or μCT) systems developed in the early 1980s had much better spatial resolution, producing voxels in the range of 5–50 μm, or approximately 1,000,000 times smaller in volume than CT voxels. With the development of Micro-CT systems, the newest generation of such systems allows for in-vivo imaging of small live animals. Micro-CT system using microfocal spot X-ray sources and high resolution detectors, allow for projections rotated through multiple viewing directions to produce 3D reconstructed images of samples. The images represent spatial distribution maps of linear attenuation coefficients determined by the energy of the X-ray source and the atomic composition of the material sample. Since the imaging process is nondestructive, the internal features of the same sample may be examined many times and samples remain available after scanning for additional biological and mechanical testing (107).

Application of Micro-CT systems has become an effective and nondestructive technique for the measurement of enamel thickness, analysis of root canal morphology and evaluation of root canal preparation, and nowadays the main application of this technique is focused on craniofacial skeletal development and structure as a nondestructive analysis of trabecular bone. A high resolution Micro-CT system has also been used in the research of bone growth and repair that facilitated quantitative 3D

measurements of trabecular bone morphology parameters such as trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), bone volume (BV), bone surface (BS), total tissue volume (TV), and trabecular bone volume fraction (BV/TV). The unique features and wider availability of micro-CT have made it the new gold standard technique for quantifying bone architecture and has stimulated a rapidly growing number of new applications (108).

Micro CT analysis has a great role in implant dentistry. While the stability of an implant is determined by the mechanical properties of the implant–bone interface and the quality of the fixation with the bone, for long time the osseointegration of the interface has been commonly evaluated by histomorphometric analysis. Morphometric indices analogous to classical histomorphometry can be calculated from micro-CT images of trabecular and cortical bone. However, histomorphometry is a destructive method, and the same specimen could not be used for other characterizations (107-109).

Micro-CT does not provide direct information on cellular function and remodeling activity, which continues to be the domain of bone histology (110). A disadvantage of histomorphometric analysis is that only a few sections per implant could be obtained and the procedures of sample preparation often result in artifacts. Micro-CT is a nondestructive, fast, and precise technique that allows measurements of trabecular and cortical bone. It can provide a spatial representation of bone formation at the implant surface and the peri-implant region up to a few microns or even better, and can evaluate both qualitative and quantitative morphometry of bone (142).

Titanium, which is a widely used material for load bearing implants, exhibits much stronger X-ray absorption than bone. During CT scanning, as titanium absorbs and scatters X-ray energy at various rates, it often causes inherent halation artifacts, which are called “partial volume effects”, which should be taken into consideration while calculating bone density about an implant surface. The image slices may be reconstructed in an arbitrary plane and imported into image analyzing software to generate quantitative measurement of the bone area. Accuracy of Micro-CT was qualitatively evaluated by comparing to standard histomorphometric data with the corresponding CT slices for the same specimen. Furthermore, as the complete digital data on the trabecular bone structure around the implant is available, it is possible to create finite-element models of the bone-implant system that model the trabeculae in detail so that mechanical stress transfer at the interface can be studied (143- 146).

## 2.5. Animal Studies

### 2.5.1. In-Vivo versus In-Vitro Studies

In-vitro testing is popular for the characterization of bone contacting materials particularly as medical researchers embrace the principles of animal use reduction. In-vitro testing can be used primarily as a first stage test for acute toxicity and cytocompatibility to avoid an unnecessary use of animals in the testing of cytologically inappropriate materials. In-vitro testing gives information regarding cytotoxicity, genotoxicity, cell proliferation and differentiation; it is more easily standardized and quantifiable than in-vivo testing. In-vitro studies are also useful for screening new materials for product quality and the release of potentially harmful additives incorporated during the manufacturing process (147).

However, in-vitro characterization is not able to demonstrate the tissue response to materials, instead being confine to the response of individual cell lines. Additionally, cellular response such as cytotoxicity due to the presence of metal ions, can vary between cell lines and passage number. In-vitro tests also overestimate the level of material toxicity and are limited to acute studies of the effects of toxicity due to the relatively short lifespan of culture cells (148).

Another important issue is that in-vitro tissue culture maintains small tissue fragments of tissue, but does not necessarily praise the architecture. In-vitro organ culture takes place in tissue or organs, which may allow some differentiation or preservation of architecture function, but when the systemic factors are absent, the lack of vascularization limits nutrients and oxygen supply and waste removal and therefore extrapolation of results to the in-vivo situation limits the model. The dynamic properties of cell culture are difficult to control in-vitro and it is difficult to recreate the appropriate cell interactions found in-vivo. One major limitations to bone culture is the lack of control of physiological loading (147).

No in-vitro cell culture system is able to produce loading that simulates in-vivo situation and currently very few ex-vivo systems are able to approach such physiological loading. For these reasons animal models are essential for evaluating biocompatibility, tissue response and mechanical function of an orthopedic or dental material prior to

clinical use in human. Specially in Implantology, animal models allow the evaluation materials in loaded or unloaded situations over potentially long time durations. Not only can the tissues in the immediate vicinity of the implants be assessed, issues in remote locations can also be studied. It should be always remembered that any animal model is only an approximation, with each animal model having unique advantages and disadvantages (147- 149).

### 2.5.2. Rabbit as an Animal Study Model

The rabbit is one of the most commonly used animals for medical research, being used in approximately 35% of musculoskeletal research studies. Rabbit handling is easy and it reaches skeletal maturity shortly after sexual maturity at around six months of age. Rabbit is an established model for investigating the stability of MSIs in dentistry and also is frequently used in Stem cell researches (149- 154).

# 3. MATERIALS and METHODS

## 3.1. Animal Study Design

### Reasons for Choosing an Experimental Animal Study Design

Development of an optimal interface between bone and orthopedic dental implants has taken place for many years. In order to determine whether a newly developed implant material conforms to the requirements of biocompatibility, mechanical stability and safety, it must undergo rigorous testing both in-vitro and in-vivo. Results from in-vitro studies can be difficult to extrapolate to the in-vivo situation. For this reason, the use of animal models is often an essential step in the testing of orthopedic and dental implants prior to clinical use in humans. One no species fulfils all of the requirements of an ideal model, an understanding of the differences in bone architecture and remodeling will assist in the selection of a suitable model for a defined research question (147).

At the cell level point of view, risk assessment of final cellular products for human use is of utmost concern. Self-renewal of undifferentiated cells represents potential for tumor formation. Certain techniques such as cellular cloning or encapsulation of cellular products are alternatives to assure safety. Many cell-based therapies will not consist of a uniform cell population. Associated accessory cells raise additional questions for potential risk as well as their physiological role after administration. Pre-clinical animal models are an important step and special care must be taken in the interpretation of pertinent safety and biological activity. A thorough appreciation of relevant advantages and limitations must be made regarding an animal model choice (119).

### Standardization and Ethics in Animal Research

International standards established regarding the species suitable for testing implantation of materials in bone, state that dogs, sheep, goats, pigs are suitable. Although rat is one of the most commonly used species in medical research, there are significant dissimilarities between rat and human bone and the limitation of size making rats unsuitable for testing multiple implants simultaneously.

**Choosing Appropriate Animal**

When deciding on the species of animals for a particular model there are several factors that should be considered.

According to Schimandle and Boden (149), animal selection factors include: cost to require and care for animals, availability, acceptability to society, tolerance to captivity and ease of housing. The "Animal Protection Acts" outline the minimum requirements in terms of housing dimensions, lighting, flooring etc. and must be complied with, when undertaking an animal study. Other factors include low maintenance care, ease of handling, resistance to infection and disease, inter-animal uniformity, biological characteristic analogous to humans, tolerance to surgery, adequate facilities and support staff and existing database of biological information for this species. In addition to these, the lifespan of the species chosen should be suitable for the duration of the study. More specifically, for studies investigating bone-implant interactions, an understanding of species specific bone characteristics, such as bone microstructure on composition, as well as bone modeling and remodeling properties are important later extrapolating the results to human situation. Finally, the size of the animal must be considered to ensure that it is appropriate for the number and size of implants chosen.

In this regard, Hazzard et al. (150) comment that within a field of study, no single animal model will be appropriate for all purposes, nor can a model be dismissed as an inappropriate for all purposes. Further more multiple model systems are likely required to establish a broad body of knowledge.

**Details Specific for Rabbit Animal**

Rabbits are small mammals in the family of Leporidea of the order Lagomoropha. Their habitats include meadows, wood, forest and grass lands. Amongst various strains, New Zealand white strains of rabbits are commonly being used for research activities. These strains are less aggressive in nature and have less health problems as compared with other breeds (152).

Histologically, rabbit long bones have a very different microstructure from human beings. In comparison with the secondary bone structure of mature human bone, rabbits have a primary vascular longitudinal tissue structure, comprising vascular canals of osteons running parallel with the long axis of the bone, surrounding the medullary

canal as well as the periosteal surface. The bone between these layers is comprised of this haversian bone, with faster skeletal change and bone turnover than humans (152).

A drawback of rabbit as an animal model for the assessment of multiple implant materials is it size limitation. The International standard for the biological evaluation of medical devices recommends a maximum of 6 implants per rabbit. Also, the size of implant which is going to be inserted is limited. Cylindrical implants are not recommended to be larger than 2 mm in diameter and 6 mm in length. The mid- femur diameter is only about 0.5 cm for a rabbit weighting 3 kg. The size of the mandible does not permit an insertion of dental implants in rabbit. Despite this, the rabbit remains as a very popular choice of species for the testing of implants materials in bone (152).

### 3.2. Stem Cell Preparation

#### 3.2.1. Harvesting Stem Cells from Rabbit Adipose Tissue and Cell Culture

All of the procedures of stem cell preparation have been taken place in Genetics and molecular diagnostic laboratory, department of Genetics and Bioengineering, Yeditepe university.

Adipose tissue is comprised of adipocytes and a heterogeneous set of cell populations including endothelial cells, endothelial progenitor cells, pericytes, and erythrocytes that surround and support them, which upon isolation are termed the stromal vascular fraction (SVF). In order to isolate ASCs, adipose cells were harvested and then minced and digested by collagenase as previously mentioned in a study by Yildiz et al. (115). Following degradation of fat tissue by collagenase at $37^{o}$ C for 40 minutes, samples were centrifuged at 300 x g for 10 minutes. Supernatant was removed and pellets were seeded in tissue culture plates (BIOFIL, TCP, Switzerland) and grown to confluency in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) PSA (10.000 units/ml penicillin, 10.000 µg/mL streptomycin, 25 µg/mL amphotericin B) (Invitrogen, Gibco, UK). Then, the cells were harvested using 0.25% (v/v) trypsin/EDTA (Invitrogen, Gibco, UK). Medium was added to inhibit the activity of trypsin followed by centrifugation at 300 x g for 5 minutes at room temperature. Further, the pellet was dissolved in fresh medium and seeded on a T-75 flask (Zelkultur Flaschen, Switzerland). The cells were incubated at $37^{0}$C and 5%

$CO_2$ in a humidified incubator. Cells from passages 3~4 were used in all experiments (Figure 3.1).

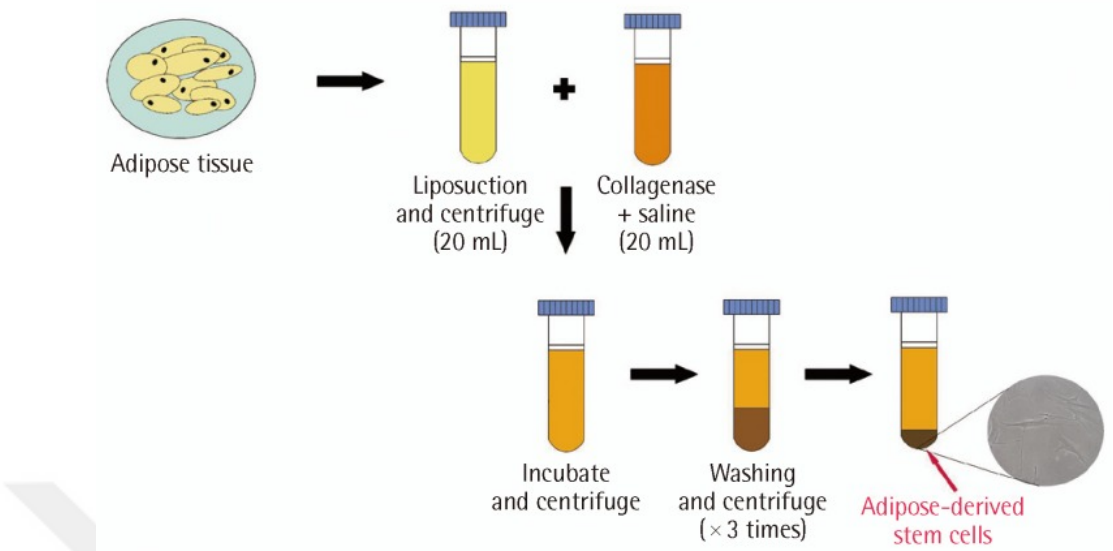


**Figure 3.1**: Schematic view of deriving stem cells from adipose tissue

### 3.2.2. Characterization of Adipose Stem Cells and Flow Cytometry Analysis

Isolated ASCs (passage 3) were characterized for their mesenchymal cell surface profile and as described previously by Tasli and Dogan (116) in 2015. Cells were trypsinized and incubated with the primary antibodies which were prepared in PBS. For characterization, primary antibodies against CD14 (ab82434), CD31 (ab27333), CD34 (ab18227), CD44 (ab58754), CD45 (ab134202), CD73 (ab157335), CD90 (ab95700), CD105 (ab53321), Integrin beta 1 (ab27314) (Abcam, UK)) and CD29 (Zymed, San Francisco, CA, USA) were used (87). The flow cytometry analysis of the cells was conducted using Becton Dickinson FACS Calibur Flow Cytometry system (Becton Dickinson, San Jose, CA, USA).

### 3.2.3. Labeling Stem Cells and Osteogenic Induction

In order to enhance osteogenic potential, ASCs were induced to differentiate into osteogenic cells according to the protocol used previously by Taşlı et al. (117). For osteogenic induction, ASCs at passage number 3-4 were seeded onto 24-well plates (Corning Plasticware, Corning, NY, cat. no. CLS3527) at a concentration of 15000

cells/well. After 24 h, osteogenic induction medium was added to ASCs. The osteogenic medium was supplemented with DMEM containing 10 % FBS (Invitrogen, Carlsbad, CA, USA), 0.1 mmol/L dexamethasone, 10 mmol/L β-glycerolphosphate, and 50 mmol/L ascorbate (Sigma Chemical Co., St. Louis, MO, USA). Cells were then incubated in a humidified incubator at 37 °C and 5 % CO2 conditions for 14 days with the differentiation media which were changed every other day.

**Von Kossa Staining**

Von Kossa staining was performed to show calcium depositions, which are markers for osteogenic differentiation. Induced cells were stained to show mineralization on osteogenic cells. Fixed cells were stained with a von Kossa kit (BioOptica, Milano, Italy) according to the manufacturer's instructions. Calcium depositions were identified using light microscopy.

**Cell Preparation for in-vivo Usage**

Osteogenicly induced cells were harvested using 0.25% (v/v) trypsin/EDTA. Medium was added to inhibit the activity of trypsin followed by centrifugation at 300 x g for 5 minutes at room temperature. Further, the pellet was dissolved in PBS. Cells were prepared as 250000 cells in 50µL of PBS for each experimental site.

### 3.2.4. Boron

In our study after consultation with Genetics and Bioengineering department, a special gel was used, with the trademark called Dermobor, which consists of % 0.2 Chlorhexidine Digluconate and %3 Sodium Pentaborate pentahidrate (NaB).

## 3.3. Implant Design

Screw-shaped titanium orthodontic MSIs made of Ti – 6A1– 4V titanium alloy; the threaded portion of the device were 1.4 mm in diameter and 6 mm in length (Osmed dental, Istanbul, Turkey). The inner part of the head of the device is reversed engineered and custom made in order to fit into the special smart peg and the outer portion of the head had four vertical flats to aid in grasping the device by holder, also 2 ditches served as an undercut for attaching the coil springs for ease of force application. (Figure 3.2)

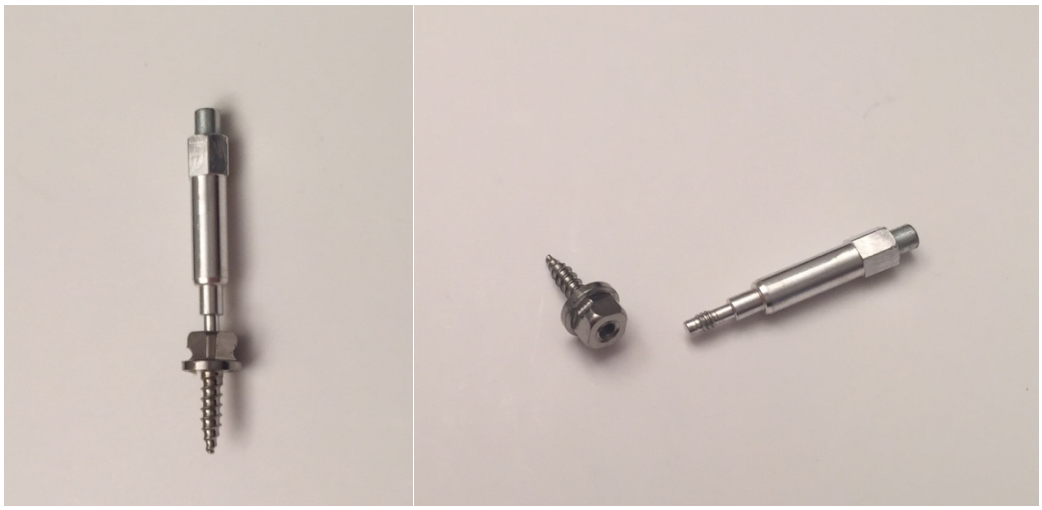

**Figure 3.2**: Mini Screw Implants fabricated for our study and smartpeg

In this study the mini implant width design was based on the previous studies, with respect to a clinical meta analysis that shows that there is no difference (P = 0.48) in TAD failure rate for mini-implants with a diameter of more than 1.3mm (12). Same meta analysis stated that there is no difference (P = 0.65) in TAD failure rate between early (less than 4 weeks) and delayed (4 weeks after insertion) orthodontic loading. The decision about follow up duration had been made accordingly. After fabrication of the mini implants, following washing with antimicrobial solutions and then normal saline, they have been sterilized in autoclave and kept in the proper packaging.

**Pilot study**

To verify the quality of the mini implant size and spotting the best position of implant insertion, a preliminary test was performed (Figure 3.3). For this purpose, a mini-implant was inserted into a fresh bone block. Afterward all the steps of the mini implant insertion were followed and the appropriate distance for implant placement was checked and activation of the coil was measured (36).

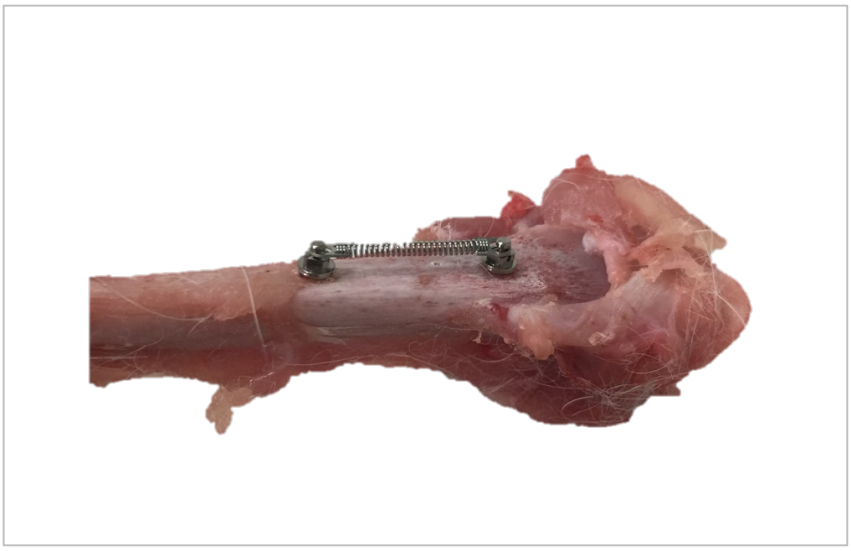

**Figure 3.3**: Insertion of mini implants in ex-vivo fresh rabbit tibia for pilot study

### 3.4. Experimental Animals

#### 3.4.1. Details of Experimental Animals

A total of fifteen white, male adult New Zealand (mean age, 6 months); rabbits weighing 3000–3500 g were used for the study. Even though all animals were raised under standardized laboratory conditions, newly arrived rabbits in the animal house were quarantined for two weeks and examined afterwards for the most common diseases. Quarantine also serve as a period of adaptation to the surroundings and the daily routine in the animal quarters. Rabbits was kept in individual mesh cages (90 × 0.60 × 0.45 m) hung at the height of 0.8 cm from the ground so that excrement can fall out into collecting trays. Fixed temperature of 23°C, relative humidity and 12 to 14 hours of light was provided for the colonies circadian biorhythms and animals were continuously observed for food consumption and fecal characteristics (Figure 3.4).

Pelleted rabbit diet (with normal nutritional levels) and distilled water were provided ad libitum. Water was changed every day and the rabbits were inspected for the signs of dehydration, since dehydration in rabbit is a very common and serious condition that needs immediate attention. Handling of the rabbits was done with care by grasping the large fold of loose skin over the shoulders with one hand and either supporting or grasping the rear feet with the other hand.

**Figure 3.4**: Standard rabbit cage

Healthy animal weighting more than 3 to 3.5 kg has a good capacity to withstand surgical trauma and leads to a better survival rate. Two rabbit with less than 2.8 kg weight was excluded from the study because of high risk in the tibia fracture during implant placement. Gender is generally not a problem but some investigators claim that female sex is biologically stronger. The rabbits in our study was chosen as male, to be in accordance with the previous studies. All experimental procedures were performed on anesthetized animals. Care was taken to avoid unnecessary stress and discomfort to the animal throughout the experimental period.

### 3.4.2. Study Groups

**Grouping and Rabbit Assignment**

A total of 15 rabbits were randomly assigned to 3 major groups, and 3 sub groups for each major group.

In A main groups the mini implants were inserted in appropriate distance and number in each animal, but no other intervention took place and they served as a control group.

In B main groups prior to implant placement, stem cells were injected into the pilot holes.

In C main groups prior to implant placement, stem cells were injected into the pilot holes and mini implants were dipped into the boron solution.

Each main group had 3 sub groups for the force application. They tiltled from 1 to 3, in which 1 meant no force, 2 assigned to 150 cN of force and 3 to 300 cN of force.

In the 2 and 3 subgroups, after insertion, the screws were immediately loaded with transverse forces. The load was provided by Sentalloys (GAC, Grafel ng, Germany) superelastic tension coil springs, tied through the heads of the implants with 0.010-in stainless steel ligature wire. The ends of the wires were tucked in below the head of the mini implant to minimize soft-tissue irritation. The coils developed a virtually constant force of 150 and 300 cN. The activation forces of the coil springs were measured with a gauge.

### 3.4.3. Pre Operative Preparation

#### 3.4.3.1. Anesthesia, Shaving and Antisepsis

Before surgery, each rabbit was placed under general anesthesia and showed no response to pain stimulation. Ketamine 50-60 mg/kg Inter Venus was administrated slowly intramuscular after the animal was immobilized. Providing inadequate Anesthesia and analgesia during surgery leads to the rabbit regaining consciousness and flocking its leg.

Ketamine is a short acting anesthetic with an induction time of half an hour during which both the legs have to be opened and implants have to be replace. Added aesthetic agent like was injected when necessary, which has proven to be safe adjunct when co-administered with Ketamine to induce short periods of surgical anesthesia. When combined with Ketamine, muscle relaxation and visceral analgesia was improved, and emergence from anesthesia is smoother. Moreover, increasing the dose does not increase the degree of sedation but rather the duration of effect.

The implantation site (tibia near the femur of the hind leg) was shaved before surgical procedure with an electric shaver and shaved hair should be cleaned away to avoid contamination. Povidone iodine and 70% ethanol was scrubbed to disinfect the surgical field before and after surgery (Figure 3.5- 3.6).

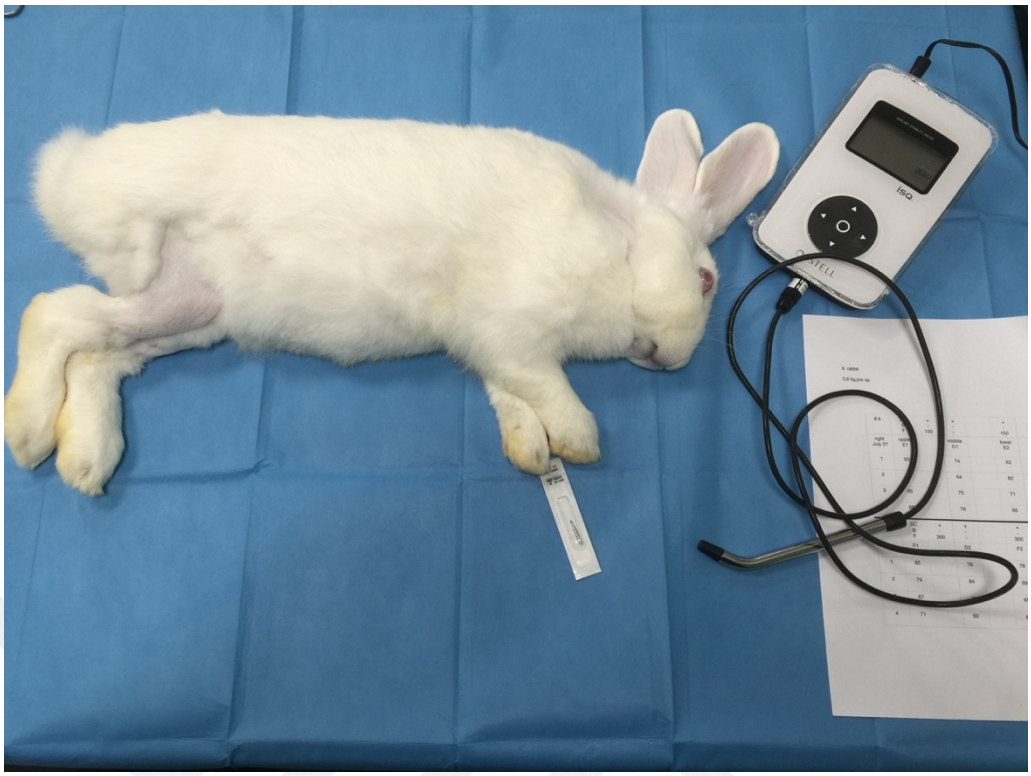

**Figure 3.5**: Shaved and anesthetized rabbit in the surgical room

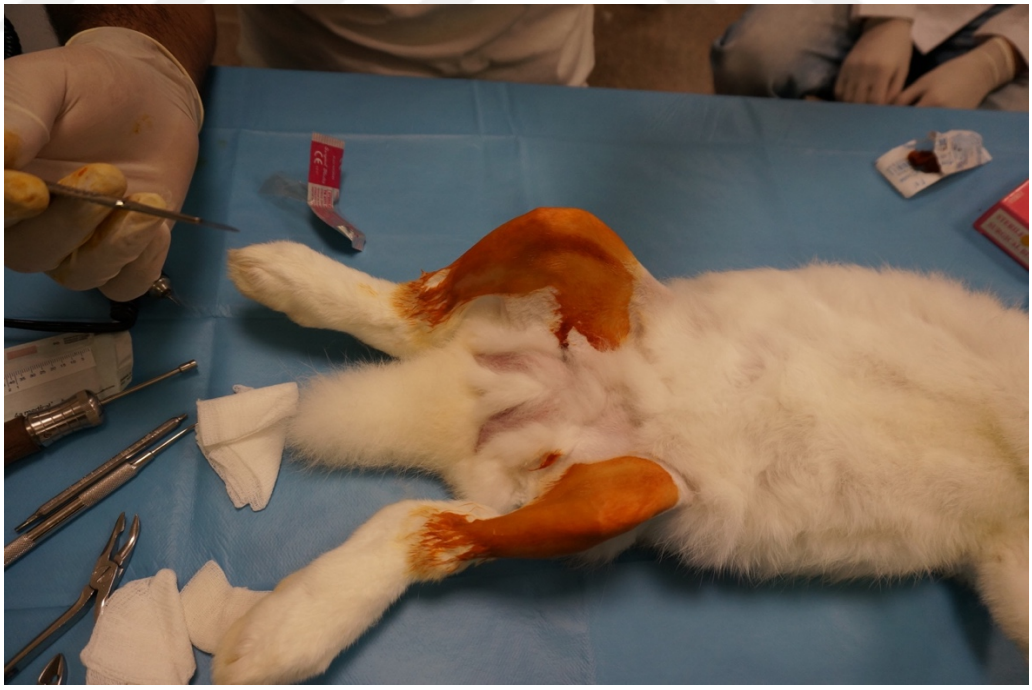

**Figure 3.6**: Antisepsis

### 3.4.3.2. Position of Implantation

Proximal femoral condyle has both cortical and cancellous bone, which provides a cushioning effect and prevents the cortical bone from splintering and give adequate space for the implementation. However, to be in accordance with the previous studies with mini-implants, that were inserted in proximal tibia condyle. By use of prefabricated distance holder, a guide drill was used first to mark the implant sites approximately 10 mm apart, with three implants on each tibia (133).

### 3.4.4. Surgical Procedure

All procedures were performed under sterile conditions in a veterinary surgical operating room. Each animal's temperature was maintained with a heating pad. The animals were sedated with an intramuscular injection of ketamine (10 mg/kg), atropine (0.06 mg/kg), and stresnil (0.03 mg/kg). In the areas exposed to surgery, 4 ml of local anaesthesia (2 per cent lidocaine with 12.5 mg/ml epinephrine, xylocain/adrenalines; Astra, Södertälje, Sweden) was injected. An incision was made using number 22 blade. All procedures for miniscrew insertion done by the same surgeon. A 3 cm incision was made on the proximal–anterior part of each tibia. The incision penetrated the epidermis, dermis, and the fascial layers, with the care not to harm any muscle (Figure 3.7).

Lateral reflection of these tissues exposed the underlying periosteum (Figure 3.8). An additional medial–anterior incision was made through the periosteum. The periosteum was elevated and retained by a self-retaining retractor, and the implants site was prepared using a pilot drill with the diameter of one mm, (Ortho Organizers, CA, USA) perforating the cortex at a low speed rotation.

Uemura et al. (97) in a study assessing orthodontic mini-implant stability and the ratio of pilot hole implant diameter, suggests that to obtain mini-implant stability, the hole diameter should be between 69 and 77 per cent of the diameter of the mini- implant for 1.3 mm diameter mini-implant. Like wise in our study 1/1.4= 71.42 falls in between this range.

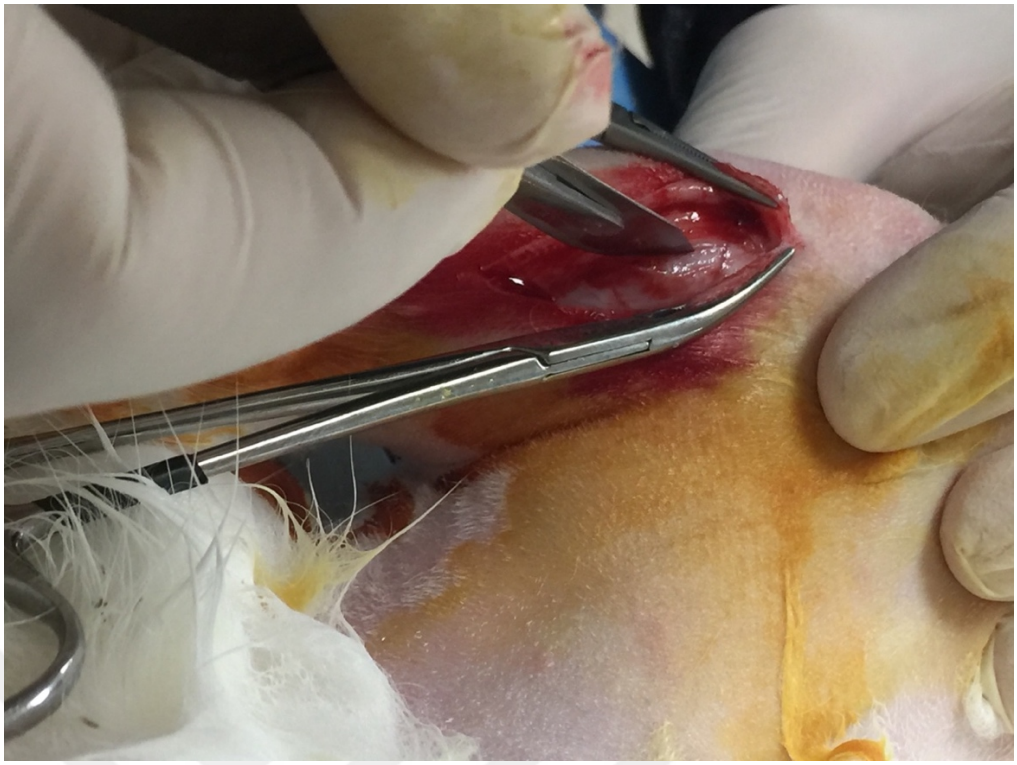

**Figure 3.7**: Incision through skin and muscle layers

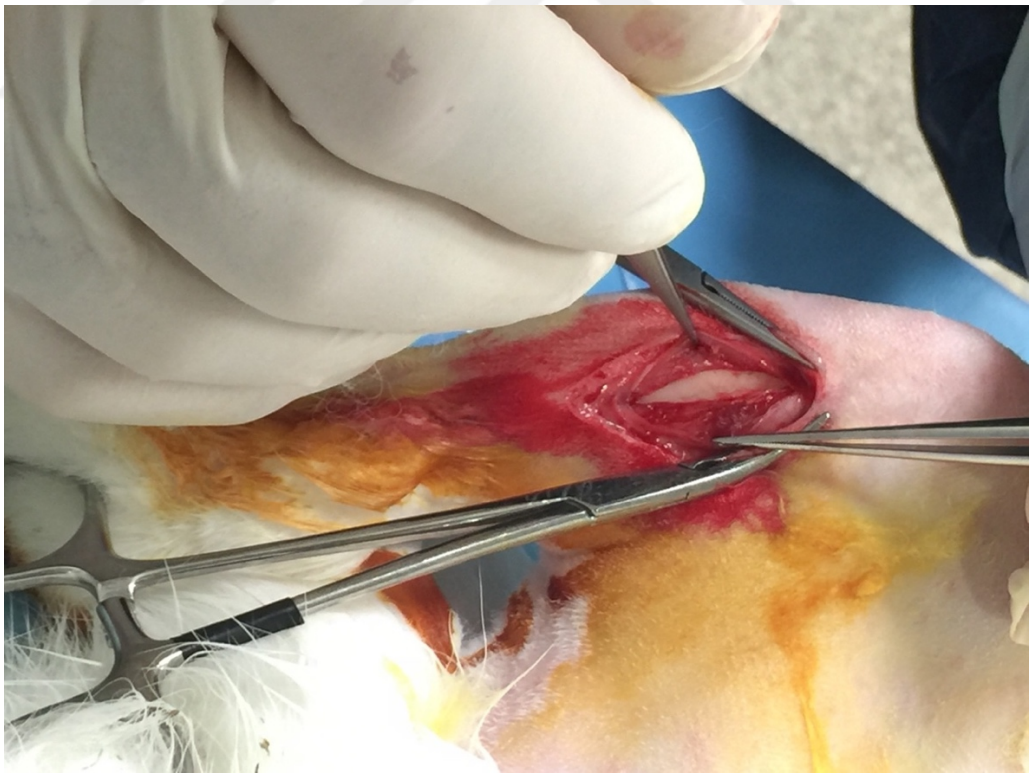

**Figure 3.8**: Retracting incised layers and reaching the bone

Drilling pilot holes, even at low speeds and under copious irrigation, can cause heating of bone beyond 47º C (71). The drilling process generates heat that impairs the turnover activity of bone tissue by causing hyperemia, necrosis, brosis, osteocytic degeneration, and increased osteoclastic activity. Bone temperature must be below 47°C during drilling to avoid thermal osteonecrosis (12). Intermittent drilling with a low speed rotary hand piece and profuse saline irrigation was performed while drilling (Figure 3.9 - 3.11).

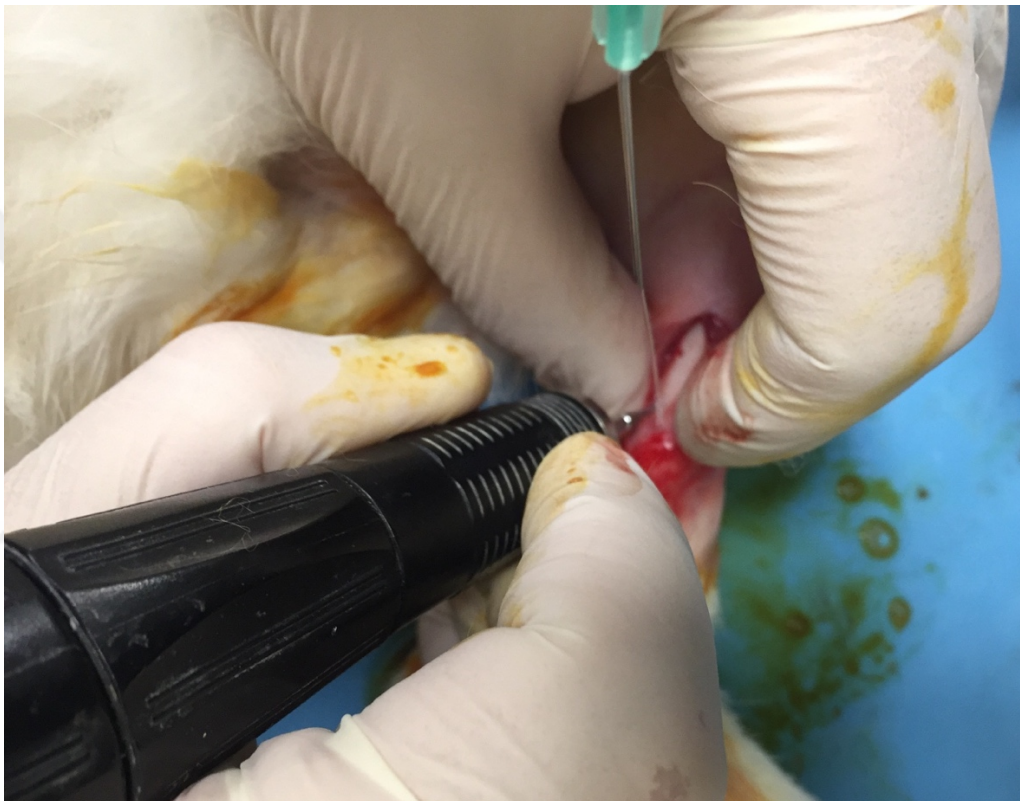

**Figure 3.9**: Intermittent drilling with a low speed rotary hand piece and profuse saline irrigation

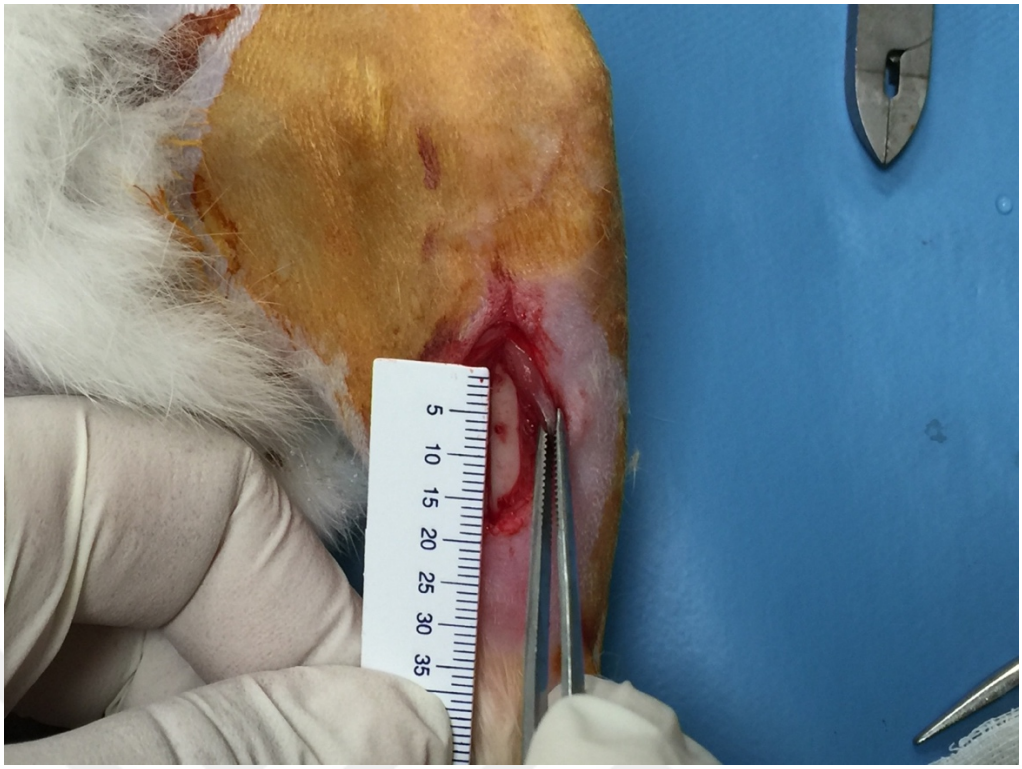


**Figure 3.10**: Measuring the distance between pilot holes

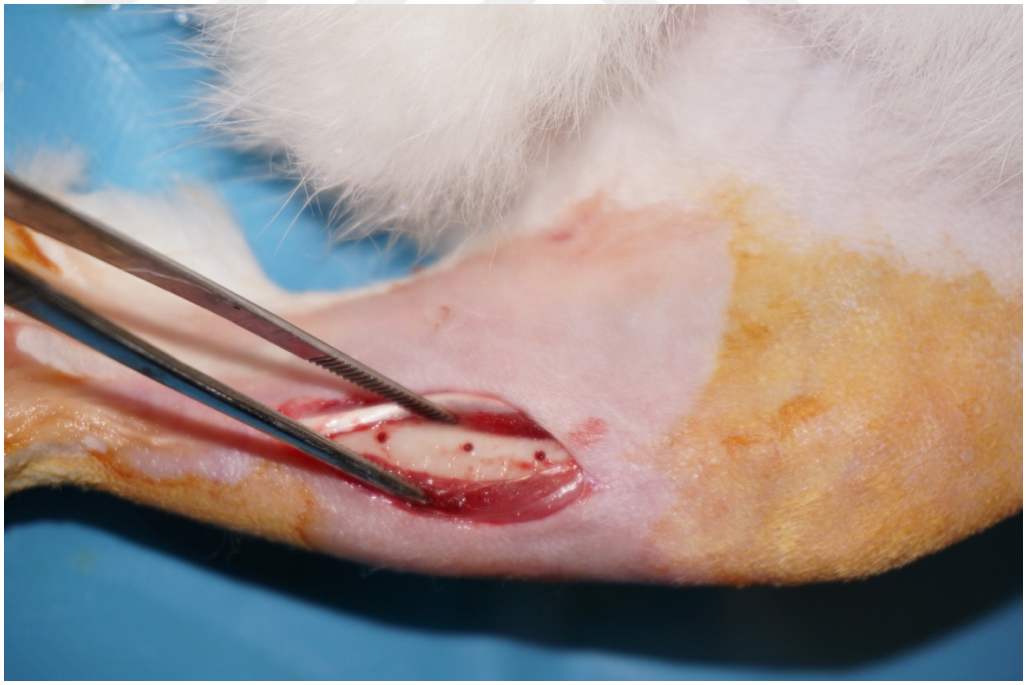

**Figure 3.11**: Prepared pilot holes in tibia

The implants were then hand inserted perpendicular to the cortical bone (Figure 3.12) and screwed slowly. Screwing was continued until the inferior part of the head of the implant came into the contact with our custom made gauge. The custom gauge consisted of a handle and a customized blade, with the thickness of 2 mm, making implant head and bone distance standard for all mini implant sites (Figure 3.13).

This distance was necessary to prevent countersink friction and overgrowth of tissue into the mini implant head (3), (Figure 3.13). Tibial bone of the rabbit was used, six implants were placed in each animal, three in each proximal tibia (Figure 3.12)

For Bor groups MSIs were immersed in the Dermobor mixture for 2 seconds and immediately inserted in the site (Figure 3.14).

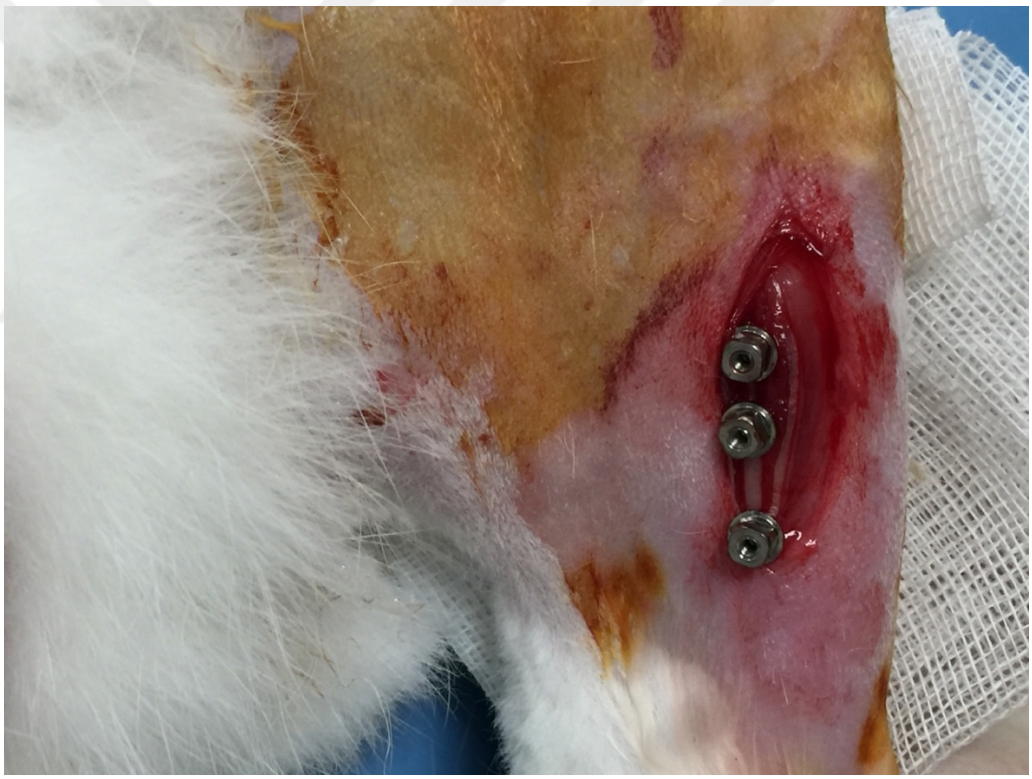

**Figure 3.12**: The implants were inserted perpendicular to the cortical bone

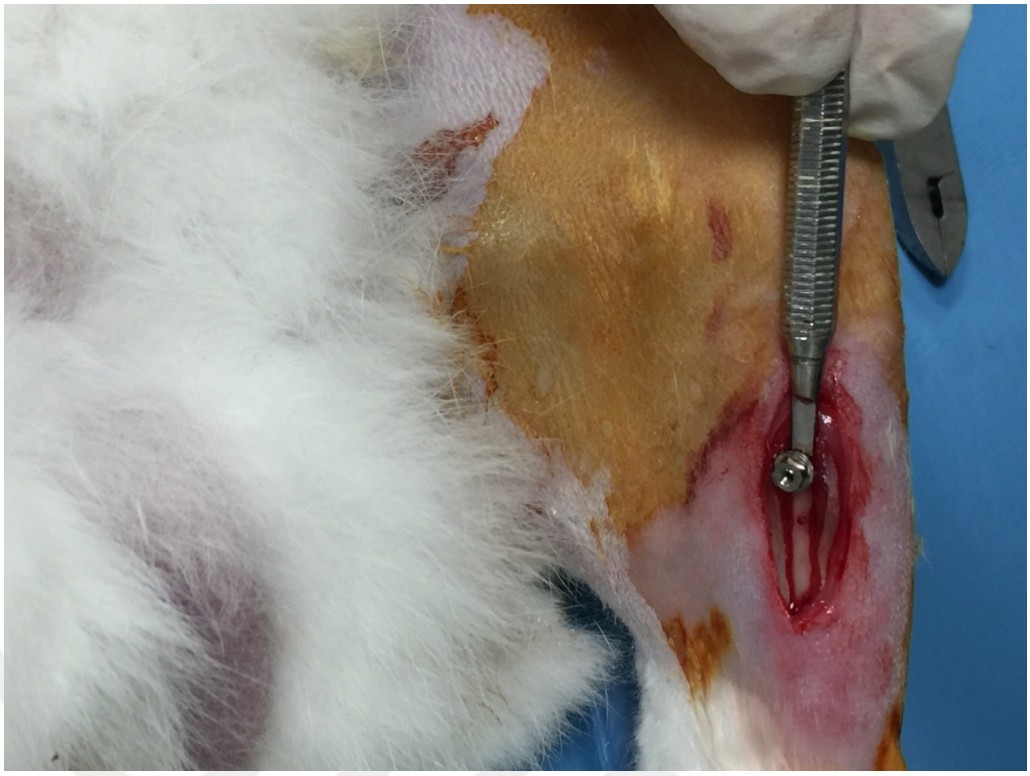

**Figure 3.13**: Making implant head and bone distance standard for all mini implant sites

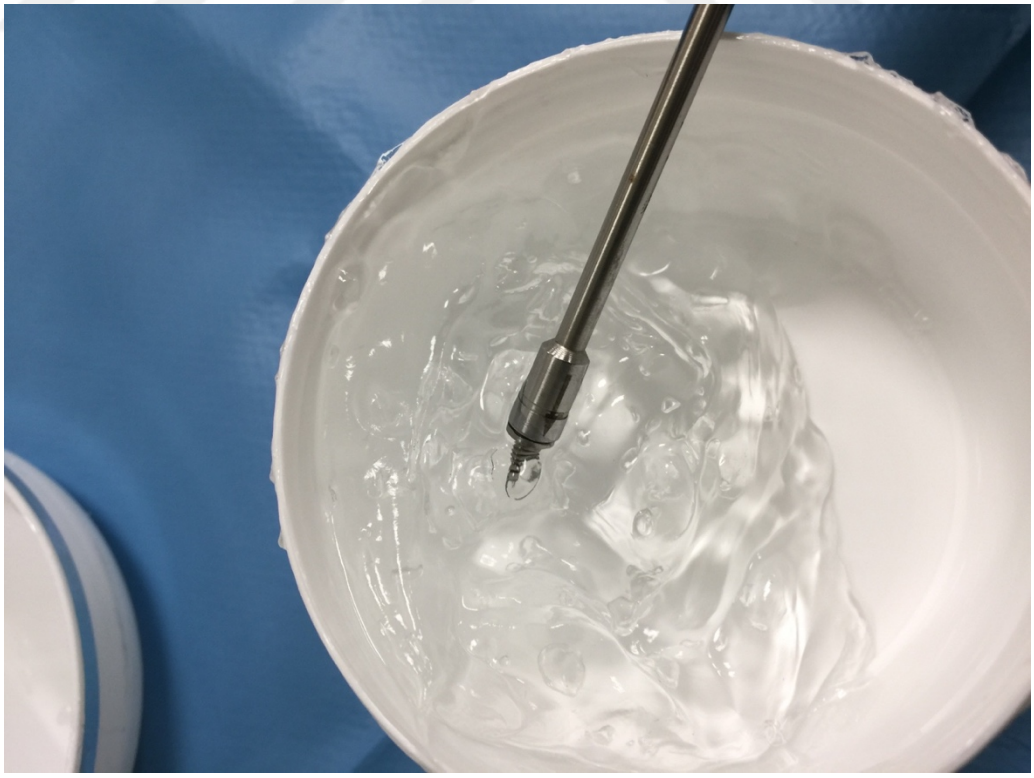

**Figure 3.14**: Dermobor

For Stem cell groups, 50µ liter of Stem cell solution, containing 250000 Stem cells were injected to the pilot holes, following immediate insertion of the mini implants.

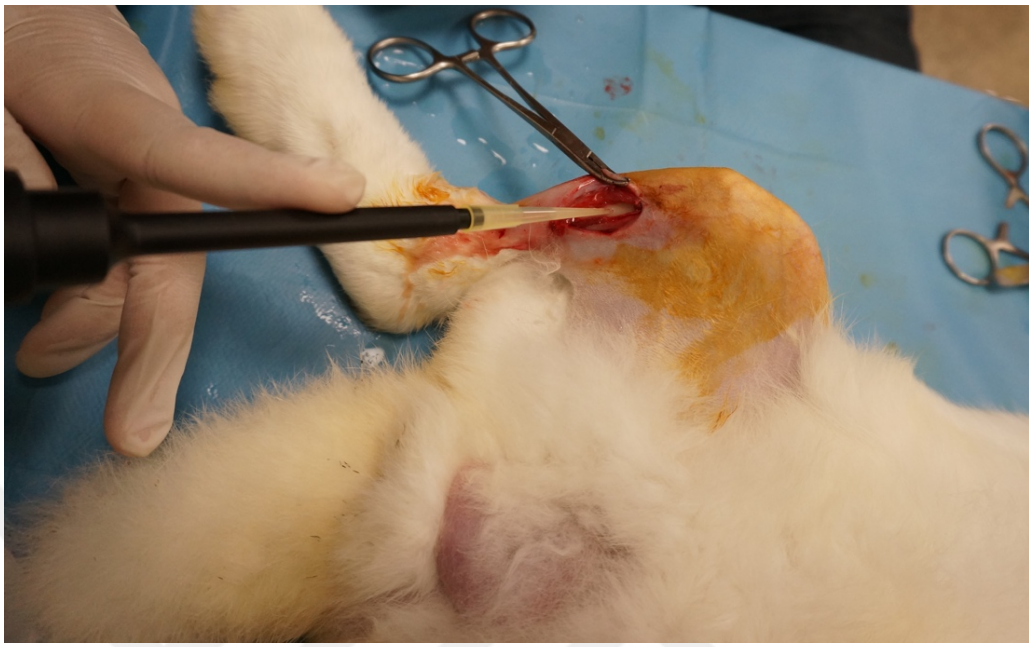

**Figure 3.15**: Injecting Stem cells into the pilot holes in Stem cell groups

Self-drilling MSIs with a large screw diameter and a tapered or conical shape might cause overcompression of the cortical bone by excessive placement torque. This can lead to microdamage, a permanent deformation of the microstructure in loaded cortical bone in the form of fatigue, creep, and eventual cracking. Therefore, the accumulation of microdamage can produce local ischemia, bone necrosis, bone remodeling, and premature loss of the implants (90).

Therefore, in this study self drilling method was avoided and any implant site that had micro fracture during implant placement had been excluded. Implants were placed bilaterally in all animals. Following these steps, smart pegs were attached to the mini implant head using hand and with meticulous care. Afterward, RFA for each mini implant sites were measured in accordance with the guidelines and previous studies for 4 times, each 90 degrees apart (36) (Figure 3.16).

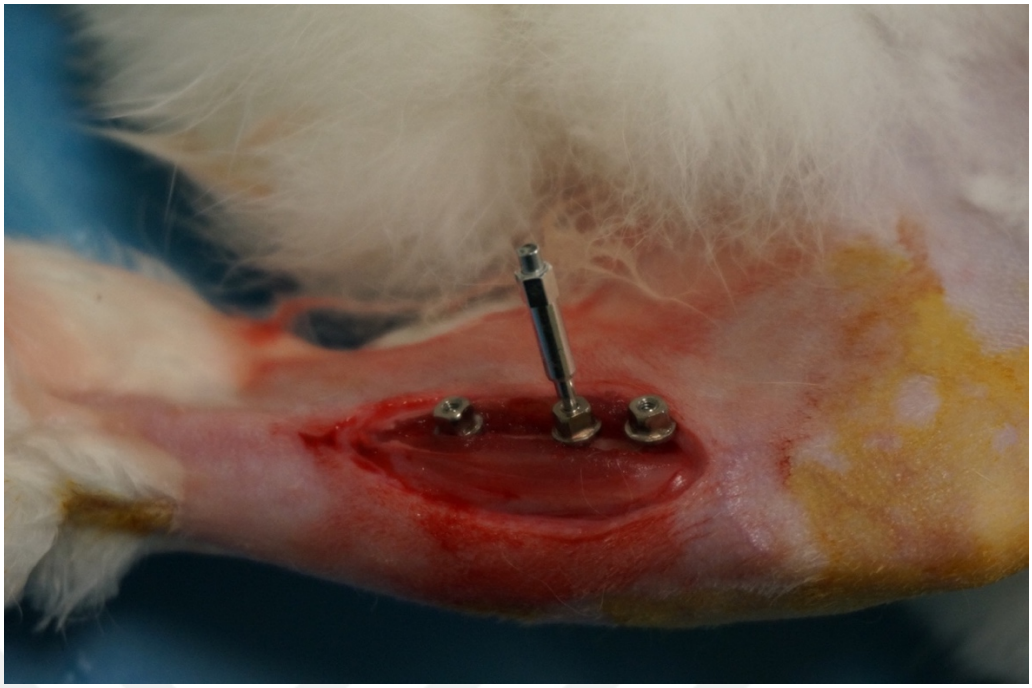

**Figure 3.16**: Smart peg attached to the mini implant head during surgical procedure

In force groups, super elastic coils were attached to the mini implant head, with wire ligatures. The activation of the coil was measured with gauge. Any impingement of the ligature ends was observed and resolved by using tucker hand instrument (Figure 3.17).

During the surgery, eyes were kept hydrated by using eye drops. The fascia and the skin were sutured separately with resorbable suture (4/0 or 3/0 cutting braided and coated poly glycolide-co-lactide surgical suture, Pegelak®, Hannover, Germany), (Figure 3.18- 3.20).

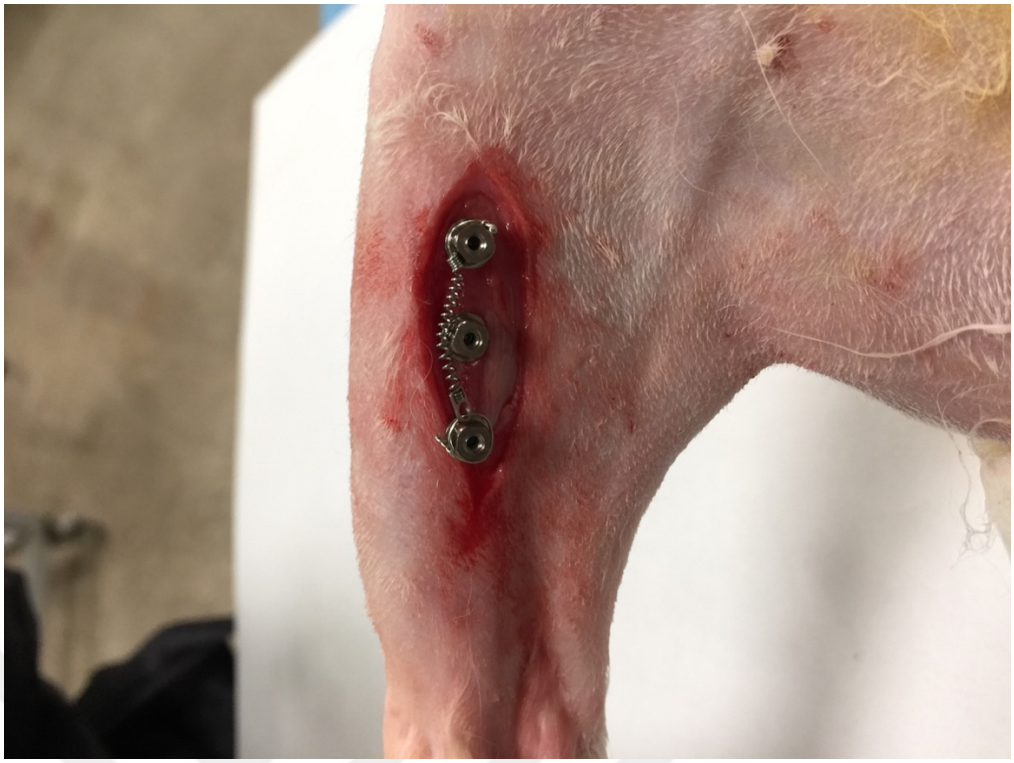

**Figure 3.17**: Super elastic coils in place

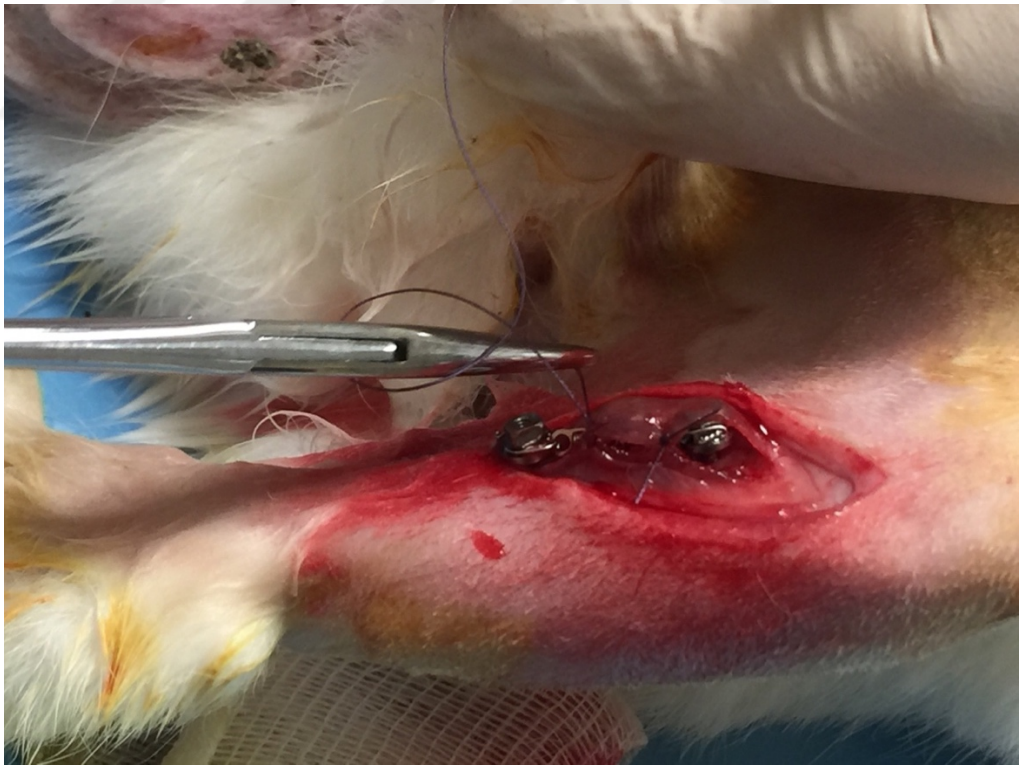

**Figure 3.18**: Suturing the surgical site

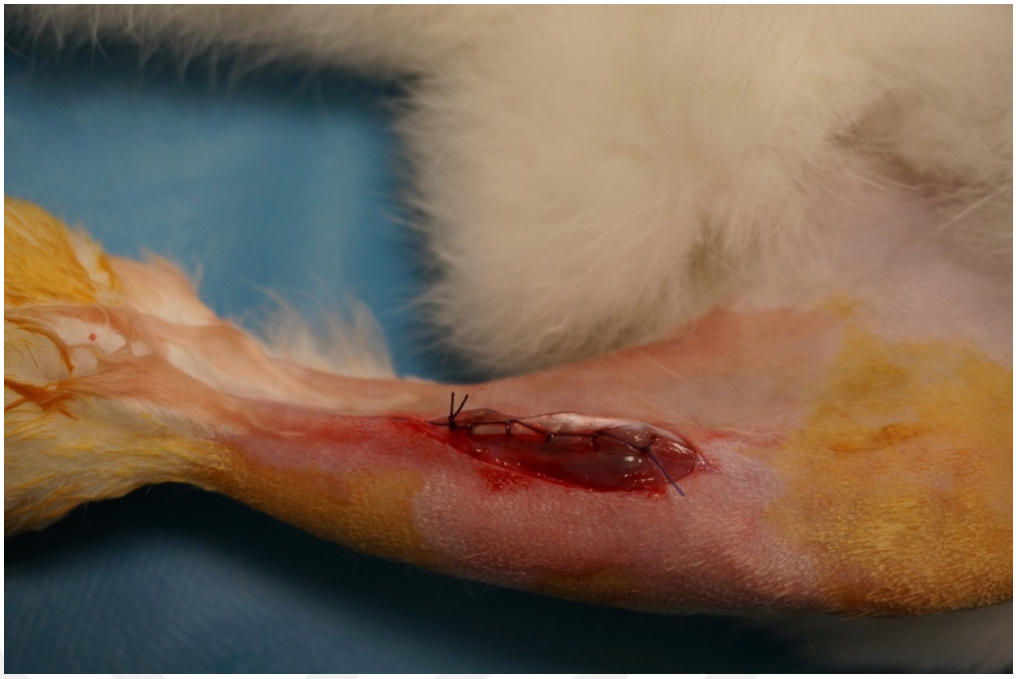

**Figure 3.19**: Suturing the fascia

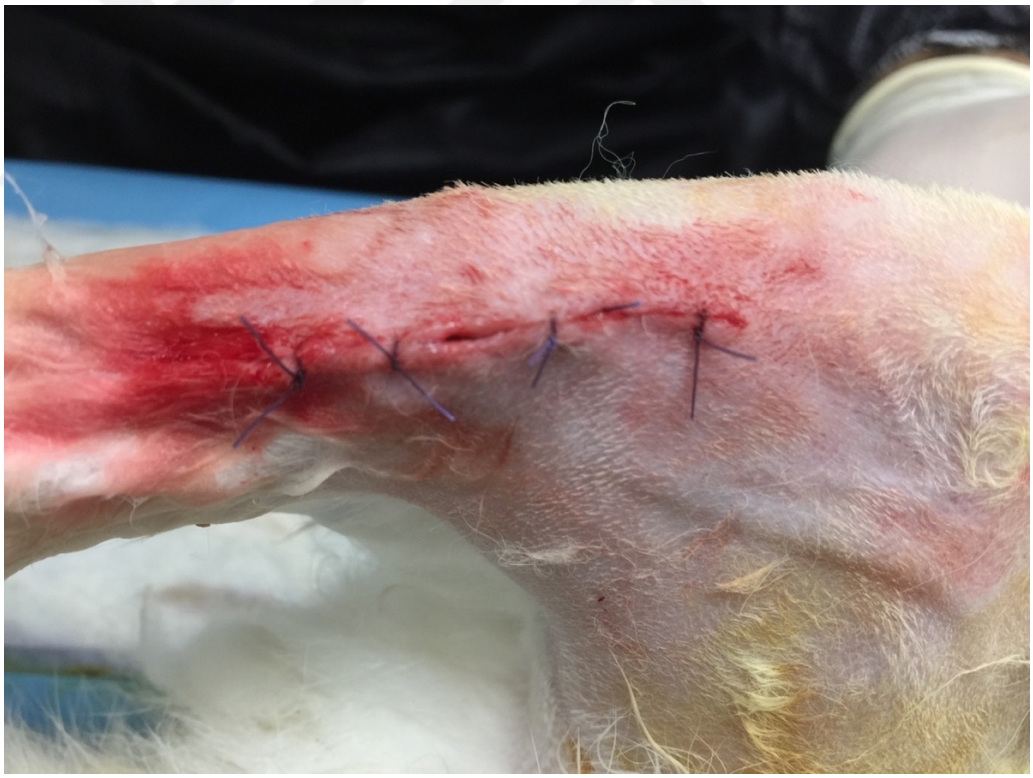

**Figure 3.20**: Suturing the skin

To prevent a rabbit from chewing the suture off, a small cotton pad was sutured on the top of the surgical wound. After one day the pad was removed gently and the area was inspected carefully by the veterinary physician (Figure 3.21).

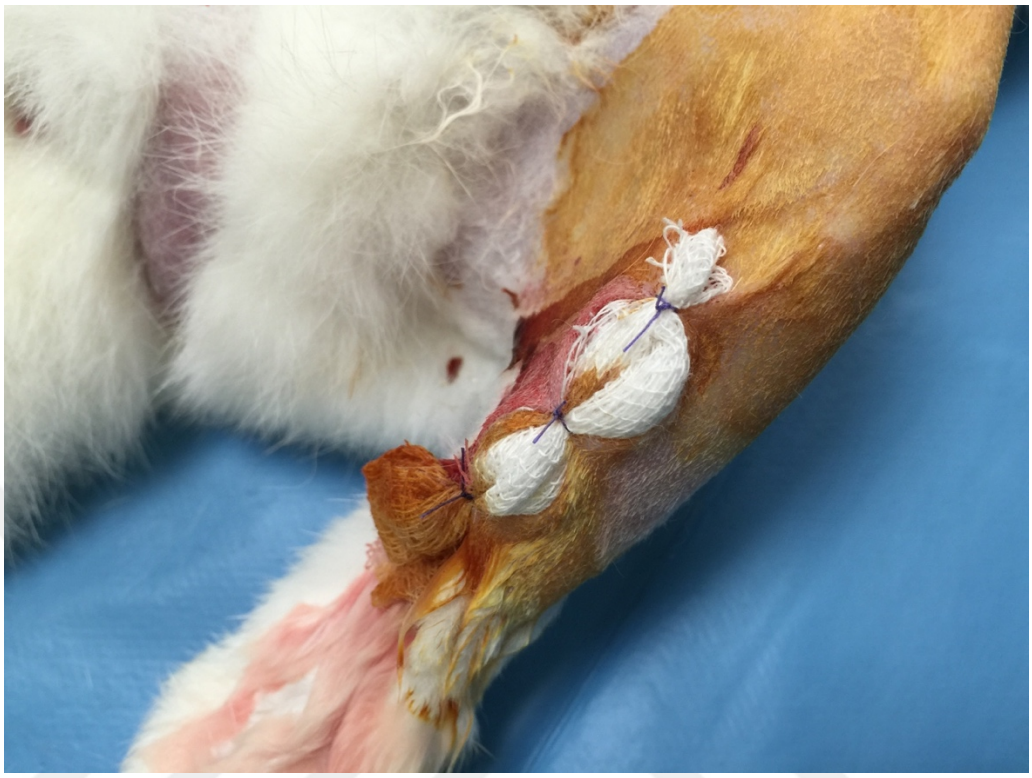

**Figure 3.21**: To prevent a rabbit from chewing the suture off, a small cotton pad was sutured on the top of the surgical wound

### 3.4.5. Post Operative Care and Follow Up

If it is considered necessary by the veterinary physician, subcutaneous antibiotic was administered (2.5 ml benzylpenicillin/ dihydrostreptomycin; Tardomycels, Bayer Vital, Leverkusen, Germany) preoperatively and then once a day for 7 days.

Moderate to severe postoperative of pain was managed to prevent serious stress to the rabbits which could severe consequences.

Animals were carefully evaluated during the first 24 post operative hours. Analgesics and anti-inflammatory drugs where administrated accordingly. The intramuscular and subcutaneous route for the pain killer was avoided as a trauma of the injection would add to that shock of the surgery.

The animals were inspected after the first few post-operative days for signs of wound dehiscence or infection and weekly thereafter to assess general health. Loading

periods of 21 days were used for the miniscrews. At day 21 after RFA measurements, all corresponding miniscrews were measured again with Osstell device. Rabbits were euthanized, immediately after that the incision was made and the ISQ values were recorded. The tibias were disarticulated and labeled. The sections were labelled using three colours of dye to code for orientation (SC versus SC+B with different F) and stored in a neutral formaldehyde in 4°C for further studies.

### 3.4.6. Euthanasia

1-week healing period in rabbits is equivalent to a 3-week period in humans (91). After 3 weeks (equivalent to about 9 weeks in humans), Euthanasia was accomplished easily, painlessly and without prior stress. The technique used in this study was CO2 (92).


**Figure 3.22**: Euthanasia with $CO_2$

### 3.5. Method of Analyses

#### 3.5.1. Resonance Frequency Analysis (RFA)

To reach a good connection between the SmartPeg and implant, the surfaces have to meet each other around implant, and they should not be too large. Since implants with many different geometries are available, it is necessary to use SmartPegs that meet these criteria and therefore a variety of SmartPeg types exist.

In our study the mini implants were used instead of dental implant. At the time of the study there was no available suitable SmartPeg or other possibility to measure on mini implants. The only possibility was having an attachment connection to use SmartPeg technique. Since the size of mini implants are relatively small, adding an attachment connection could compromise the stability and clinical effectiveness of them. More over in contrast to the dental implants, attachment is not an integral part of the mini implant, and any kind of adhesive material, such as curing plaster would result in difference ISQ values. A unique customized mini implant was designed with a built-in head, precisely architectured and, completely adaptable to one type of the SmartPeg that the company proposed us due to its length, thread type, diameter and connection surfaces. SmartPeg type 53, Art. No. 100484.

For dental implants high stability range is $>$ 70 ISQ, between 60-69 is medium stability and $<$ 60 ISQ is considered as low stability. Comparing absolute values between implant systems is not recommended or encouraged. Relative ISQ values is a different issue; since the difference between SmartPeg types is made as small as possible, it is correct to compare relative values; i.e. the change in ISQ from one point in time to another.

**Designing new SmartPegs**

When a SmartPeg for a new implant is designed, it is adapted to fit to the geometry of the implant, have the right thread and to be clinically user-friendly. The company make a new SmartPeg to give comparable or a similar ISQ results for each system. By using this method, it ensures that two different implants will not deviate too much in ISQ, given the same stability. In this study the SmartPeg was reversed engineered, to customize the mini implants, keeping in mind that the ISQ values will be

different from those absolute values commonly seen with dental implants, and only relative values and also changes in values over time should be the focus of this study.

SmartPeg are made from soft Aluminum with a Zinc coated magnet mounted on top of it. The SmartPegs will therefore rapidly wear after being opened. For this reason and for hygienic reasons, the SmartPeg are disposable. In this study one SmartPeg has been used for a single surgical procedure of each rabbit, after the surgery the SmartPegs were disposed subsequently.

The sterile package that holds the SmartPeg will hold strerility at least five years. During this time, it is possible that some areas on the SmartPeg will shift in color, the spots are an oxide and will not affect the function or sterility.

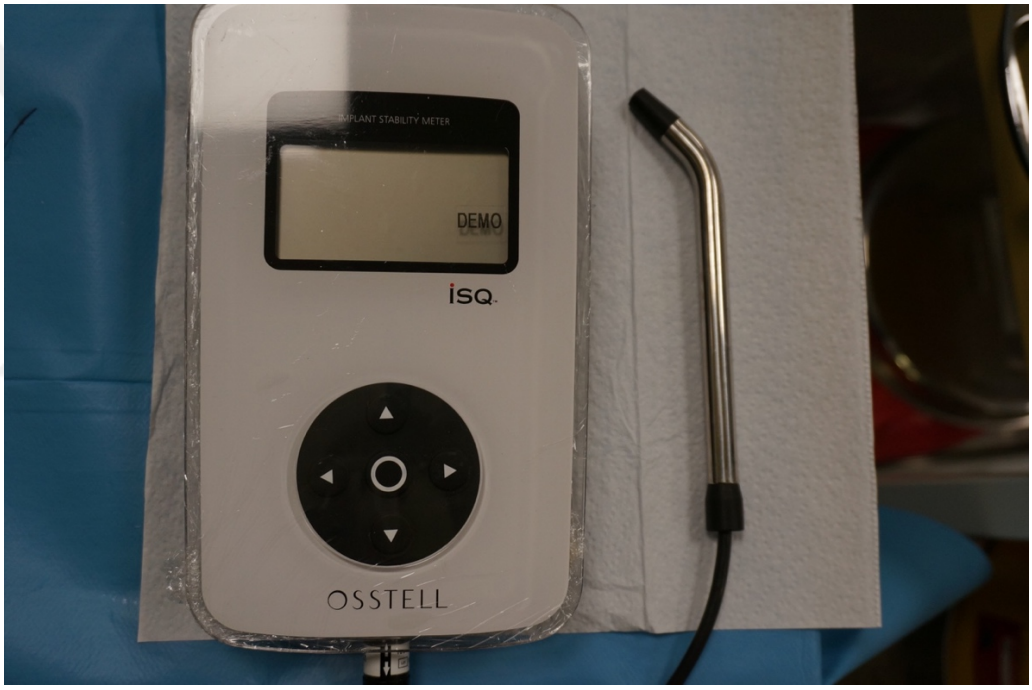


**Figure 3.23**: The Osstell device in surgery

In this study the Osstell's transducer was oriented perpendicular to the long axis of MSI (Figure 3.24), and four blinded implant stability quotient measurements were made around the MSI, 90° apart. The 4 measurements were averaged (Figure 3.25). Each MSI had 2 sets of measurements; the initial ones, taken at placement of the MSI and 3 weeks post operatively (3).

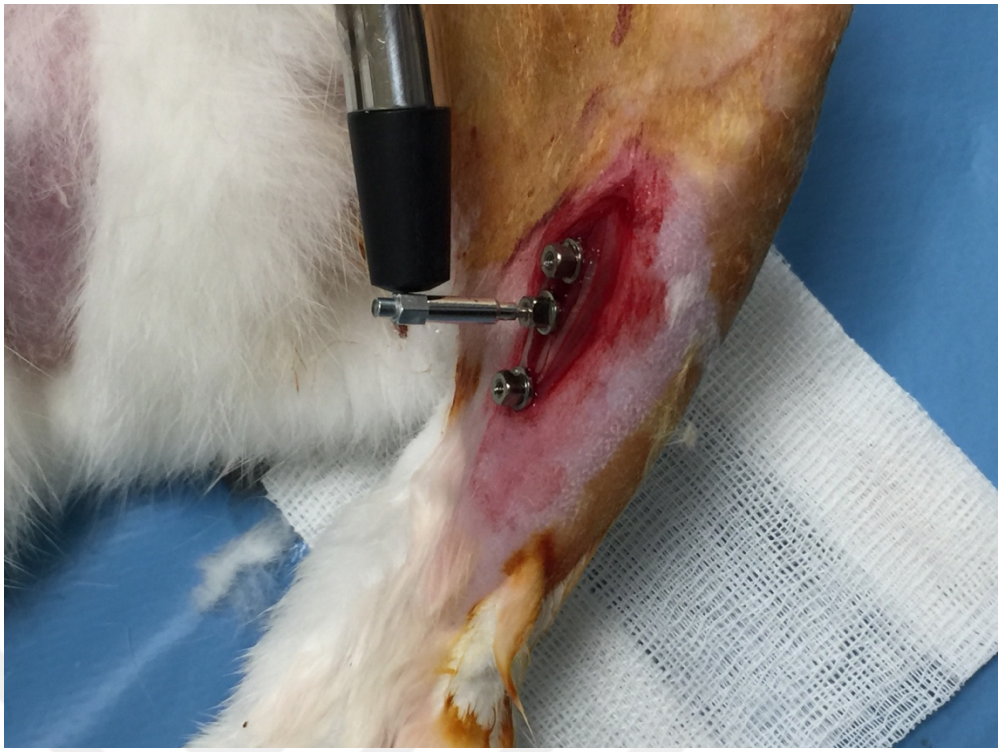

**Figure 3.24**: Osstell's transducer is oriented perpendicular to the long axis of MSI during surgery

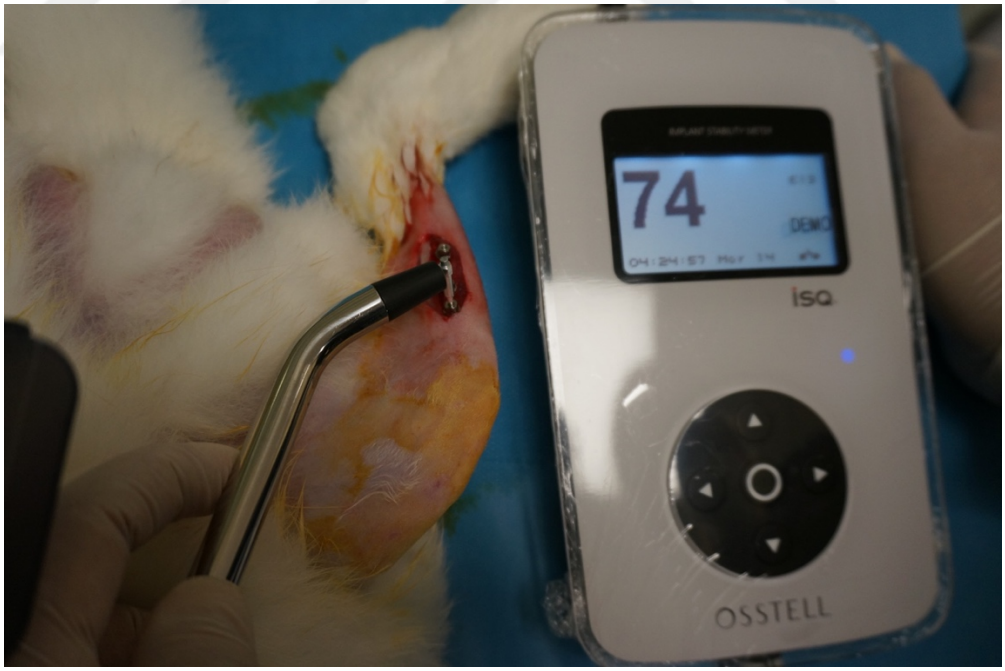


**Figure 3.25**: Recording the RFA measurements

The primary outcome value was the change in ISQ from the mean baseline measurement for each miniscrew. All measurements were carried out by one blinded investigator.

When measuring the environment should not contain an alternating magnetic field, like mobile phones or televisions. The manufacturer suggests the torque device is not needed to tighten the SmartPeg, finger tightening (4-5 Ncm) give a firm contact between the components.

### 3.5.2. Micro-Computed Tomography (µCT)

The potential bone–implant contact area is important for mechanical interlocking in establishing primary stability and osseointegration in secondary stability (23, 143).

Although information on surface area would have been valuable, this parameter is very difficult to calculate using mathematical methods alone because of the complexity of mini-implant design features such as threading and taper. Hong et al. (144) used micro-computed tomography (µ-CT) to precisely compute the mini- implant surface area engaged in cortical and trabecular bone layers for different implant designs. The use of µ -CT has been proven to be an accurate and reproducible method of quantifying microstructures in the field of biomedical and material research.

It was needed to ensure the precise fit into the plate (3.8-mm internal diameter) sample holders used for scanning, a wet paper towels and plastic paraffin film was used to prevent drying during scan of the bone specimens. Each bone-implant specimens were placed in each holder, containing three MSIs, and kept hydrated with wet paper towels. The samples were evaluated using micro computed tomography (SkyScan 1172; Bruker Cooperation, Kontich, Belgium) with an isotropic resolution of 25.9 µm. X-ray energy levels were set to 100 kV, current to 100 mA, and integration time to 520 ms. A 0.5-mm Aluminum and 0.5-mm Copper filter and a high resolution setting of 720 projections per 360° were used to ensure the highest quality scans with minimal metal implant artifact (144). The average scanning time was approximately 55 minutes per specimen (Figure 3.26).


**Figure 3.26**: SkyScanner model 1172

To be able to eliminate all metal artifacts, the reconstruction has been done carefully with NRecon software (v. 1.6.9.4) and reconstruction parameters have been chosen according to each scan optimally using modified Feldkamp's back-projection algorithm. Dataviewer (version: 1.5.1) software is used to save rotated cross-sectional images in Cartesian coordinate system in-line with MSIs direction. Reconstructed bone specimens, containing grayscale values for each voxel (3D pixel), have been segmented (binarized) by using peak-valley method and analyzed via CT-An (CT-Analyzer, version: 1.14.4.1) program. For consistency of data, the range of the threshold for specimens was kept constant for all specimens.

Binarized image analysis have been done with selecting ROIs (region of interests) around miniscrews and then analyzed. Miniscrews are eliminated by segmentation and the surrounding bone specimen have been investigated according to post-processing parameters such that BV/TV (bone volume to total volume ratio), closed porosity, structure thickness and structure separation (Figure 3.27 a-c).

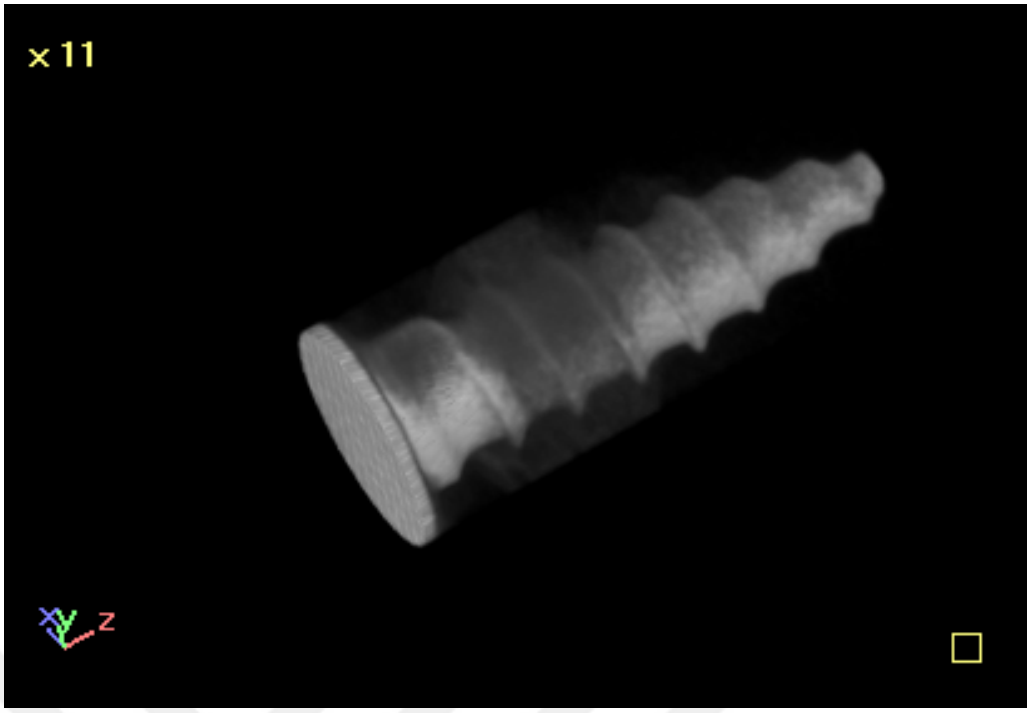


**Figure 3.27 a**: Gray scale volume of interest in mirco Computed Tomography analysis

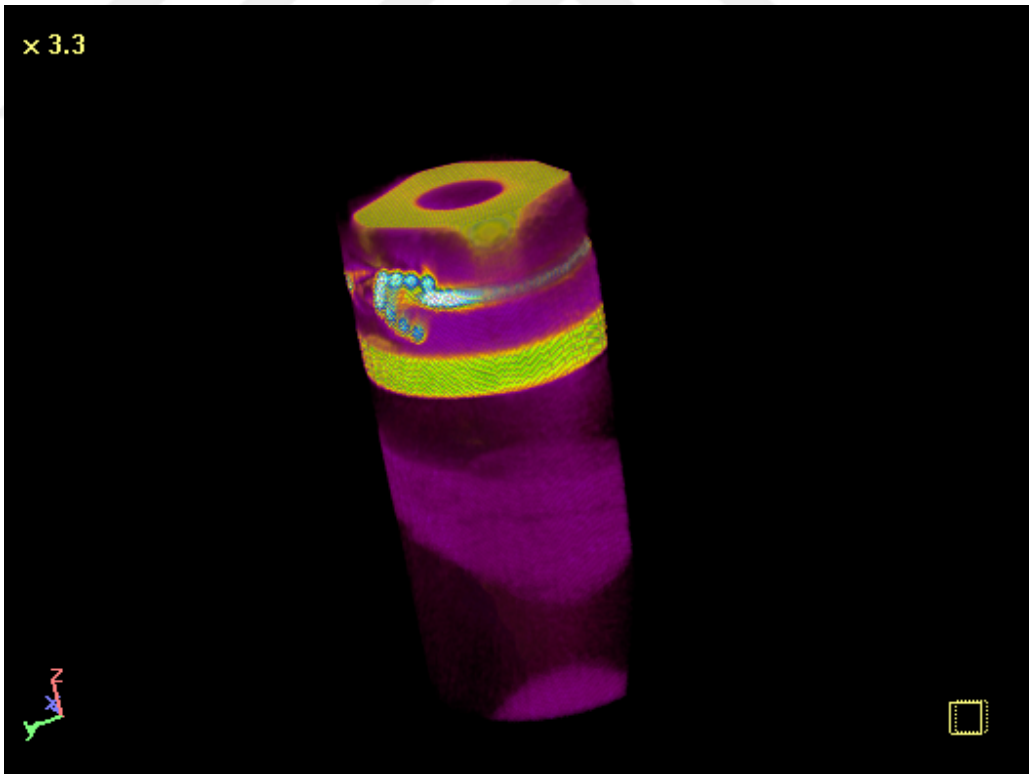


**Figure 3.27 a**: Magnified reconstructed volume of interest in mirco Computed Tomography analysis

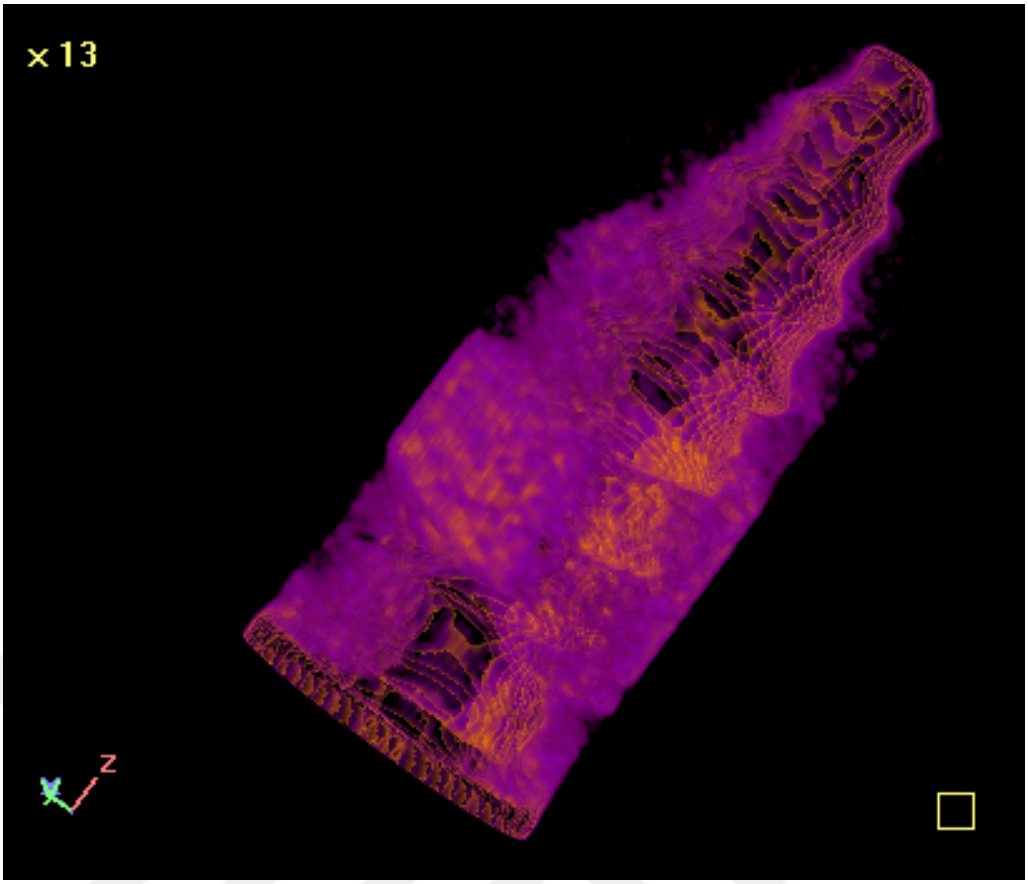


**Figure 3.27 b**: Three-dimensional images of a bone-implant specimen constructed for the defined threshold values

### 3.6. Statistical Analysis

#### 3.6.1. Priore Power Analysis

Calculation was performed using G*Power version 3.0.10. For sample size calculation data of a prospective animal study by Uysal et al. (5) on 2012 was used. This study evaluated the effect of light-emitting diode (LED) photobiomodulation therapy (LPT) on the stability of immediately loaded miniscrews under different force levels, as assessed by resonance frequency analysis (RFA). The study design and follow up of the study was nearly the same as our study. Statistically significant differences were found for changes in (ISQ) values between LED-LPT group and the control in the mentioned study. Consequently; for Power Analysis of the current study, using $\alpha = 0.05$, $(1-\beta) = 0.85$ and large effective size 1.5, it has been calculated that at least 8 samples is required in each group, considering total of 9 groups in this study, and the maximum mini implant insertion in each rabbit as 6, or occasionally 4, with the mean of 5, the total of 15 animals is required to perform this study with the mentioned statistical standards. Consequently, total of 15 rabbits were recruited in this study.

#### 3.6.2. Statistical Analysis

All data were analyzed with the statistical package for social sciences, 25.0 (SPSS for Windows; SPSS Inc., Chicago, Illinois, USA). Descriptive statistics were given as quartiles [25th, 50th (median) and 75th], mean and standard deviation. The normality test of Shapiro-Wilk's test was applied to the response variable ISQ (with values between 0 and 100 like a percentage), body weight, micro CT measurements. The data were not normally distributed. Thus, the statistical evaluation was performed using non-parametric tests. All baseline values and sub-group differences were compared by Kruskal-Wallis test. When the p-value was less than 0.05, the statistical test was determined as significant.

# 4. RESULTS

No rabbit experienced infection or significant weight loss. After 3 weeks, all MSIs were stable with no mobility.

Table 4.1 shows the Experimental design; A stands for "No SC", B stands for "SC", C stands for "SC and Boron". For Force sub groups, 1 is assigned to 0 force group, 2 is assigned to 150 cN force, and 3 is assigned to 300 cN force. No statistically significant changes in body weight were observed between groups during study (Table 4.2). The baseline ISQ values of all groups were compared and found no statistically significant differences (Table 4.3). Thus, it revealed that initial primer stability of all miniscrews was similar in all groups at the start of the experimental procedure.

**Table 4.1:** Experimental design (A stands for No SC, B stands for SC, C stands for SC and Boron); (1 is assigned to 0 force group, 2 is assigned to 150 cN force, and 3 is assigned to 300 cN force).

| Number of Animal (n= 15) | Group | Number of MIS (n=77) | SC | Boron | Force (cN) | Observation period (days) |
|---|---|---|---|---|---|---|
| A=6 | A1 | 13 | _ | _ | 0 | 21 |
| B=4 | B1 | 6 | + | _ | 0 | 21 |
| C=5 | C1 | 10 | + | + | 0 | 21 |
| | A2 | 8 | _ | _ | 150 | 21 |
| | B2 | 6 | + | _ | 150 | 21 |
| | C2 | 10 | + | + | 150 | 21 |
| | A3 | 8 | _ | _ | 300 | 21 |
| | B3 | 6 | + | _ | 300 | 21 |
| | C3 | 10 | + | + | 300 | 21 |

**Table 4.2:** Body weight changes (kg) between groups (A stands for No SC, B stands for SC, C stands for SC+Boron)

| Group | n (Rabbits) | T1 (Baseline) | | T2 (End of observation period) | | T1-T2 | |
|---|---|---|---|---|---|---|---|
| | | Mean (kg) | SD | Mean (kg) | SD | Mean (kg) | SD |
| A | 6 | 3.53 | ±0.37 | 3.55 | ±0.48 | -0.01 | ±0.13 |
| B | 4 | 3.60 | ±0.14 | 3.59 | ±0.25 | 0.13 | ±0.13 |
| C | 5 | 3.60 | ±0.34 | 3.70 | ±0.38 | -0.11 | ±0.12 |
| p value* | | 0.844 | | 0.734 | | 0.184 | |

*p values were calculated from Kruskal-Wallis test, $p < 0.05$

**Table 4.3:** Descriptive statistics and comparisons of **baseline** ISQ values in three groups (control and SC and SC+Boron).

| Group | n (MSI) | SC | Boron | Force(cN) | Baseline (T1) Median ISQ | p value* |
|---|---|---|---|---|---|---|
| A1 | 13 | _ | _ | | 76.00 | |
| B1 | 6 | + | _ | 0 | 75.50 | 0.135 |
| C1 | 10 | + | + | | 71.25 | |
| A2 | 8 | _ | _ | | 74.50 | |
| B2 | 6 | + | _ | 150 | 74.25 | 0.779 |
| C2 | 10 | + | + | | 75.75 | |
| A3 | 8 | _ | _ | | 76.87 | |
| B3 | 6 | + | _ | 300 | 73.62 | 0.498 |
| C3 | 10 | + | + | | 74.75 | |

*p values were calculated from Kruskal- Wallis test, $p < 0.05$

Descriptive statistics and comparisons of ISQ values at the end of observation period under different force levels in each sub groups were shown in were shown in Table 4.4 and descriptive statistics and comparisons of changes in ISQ values during observation period under different force levels in each sub groups and sub group comparisons were shown in Table 4.5. According to Independent samples Kruskal Wallis test, at the end of observation period ISQ values of all miniscrews were found lower than the baseline values in all sub groups at the end of the observation period except for C1 with 300 cN force and no SC or Boron therapy, although no statistically significant changes were determined in ISQ values of any individual sub groups. According to Independent samples Kruskal-Wallis test, no statistically significant changes were determined in ISQ values of any force groups, under 0 cN ($p = 0.057$), 150 cN ($p = 0.893$), and 300 cN ($p = 0.972$) force levels.

**Table 4.4:** Descriptive statistics and comparisons of ISQ values at the **end of observation period** under different force levels in each sub groups

| Group | n (MSI) | SC | Boron | Force (cN) | End of the observation period (T2) Median ISQ | p value* |
|---|---|---|---|---|---|---|
| 1 | 13 | _ | _ | | 73.00 | |
| 2 | 6 | + | _ | 0 | 72.62 | 0.900 |
| 3 | 10 | + | + | | 74.12 | |
| 4 | 8 | _ | _ | | 72.00 | |
| 5 | 6 | + | _ | 150 | 69.75 | 0.505 |
| 6 | 10 | + | + | | 74.25 | |
| 7 | 8 | _ | _ | | 72.25 | |
| 8 | 6 | + | _ | 300 | 70.00 | 0.545 |
| 9 | 10 | + | + | | 71.50 | |

*p values were calculated from Kruskal-Wallis test, $p < 0.05$

**Table 4.5:** Descriptive statistics and comparisons of **changes** in ISQ values during observation period under different force levels in each sub groups and sub group comparisons

| Group | n (MSI) | SC | Boron | Force (cN) | Median ISQ Difference (T2−T1) | p value* |
|---|---|---|---|---|---|---|
| A1 | 13 | _ | _ | | 2.75 | 0.055 |
| B1 | 6 | + | _ | 0 | 3.12 | 0.249 |
| C1 | 10 | + | + | | -3.62 | 0.169 |
| | | | | | | 0.057 |
| A2 | 8 | _ | _ | | 4.00 | 0.161 |
| B2 | 6 | + | _ | 150 | 3.50 | 0.249 |
| C2 | 10 | + | + | | 1.12 | 0.359 |
| | | | | | | 0.893 |
| A3 | 8 | _ | _ | | 0.50 | 0.362 |
| B3 | 6 | + | _ | 300 | 2.62 | 0.345 |
| C3 | 10 | + | + | | 2.50 | 0.262 |
| | | | | | | 0.972 |

*p values were calculated from Kruskal-Wallis test, $p < 0.05$

Figures 4.1 demonstrates the pre- and post- stability ISQ values while no force was applied. Figure 4.2 and 4.3 shows the pre- and post- stability ISQ values while applied force was 150 cN and 300 cN respectively.

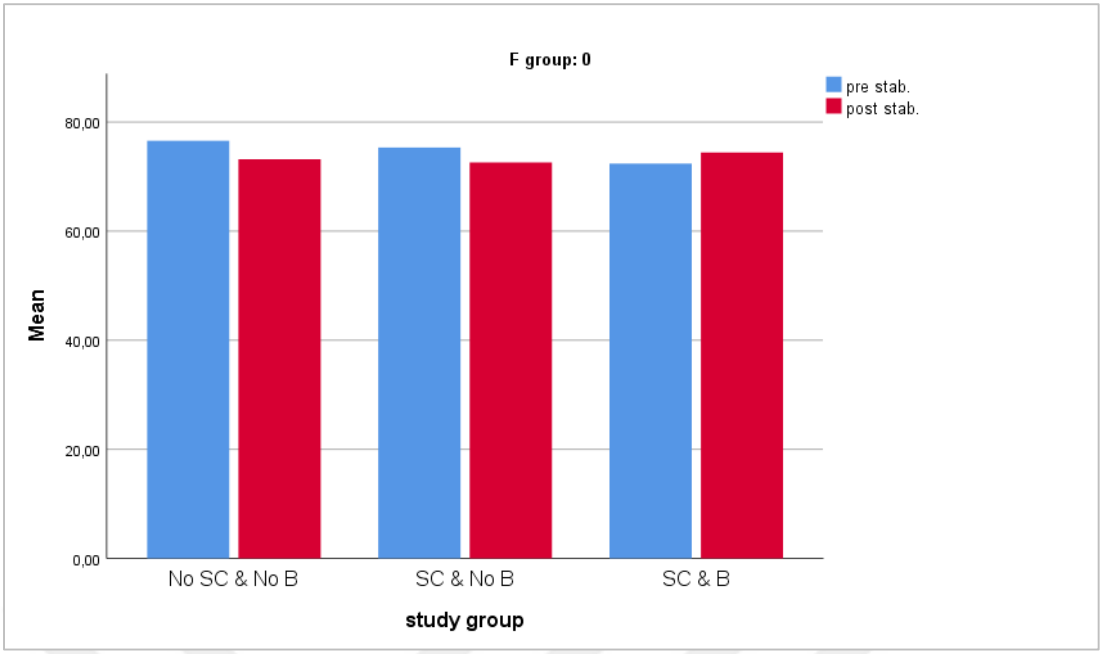


**Figure 4.1**: Graphic representation of the pre- and post- stability ISQ values while no force was applied.

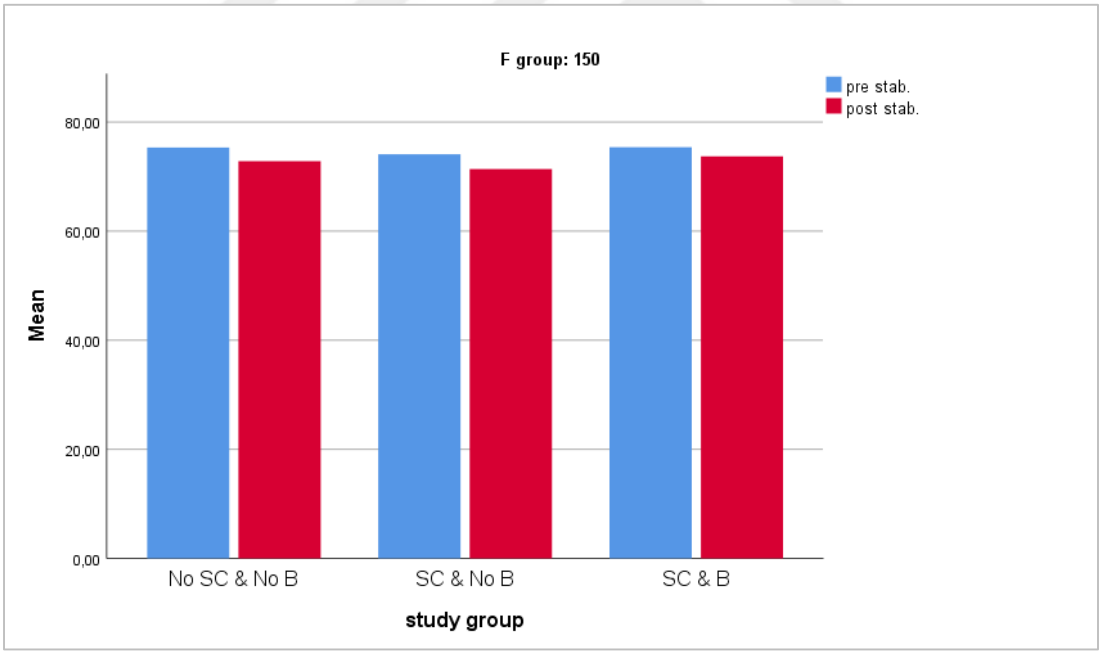


**Figure 4.2**: Graphic representation of the pre- and post- stability ISQ values while applied force was 150 cN.

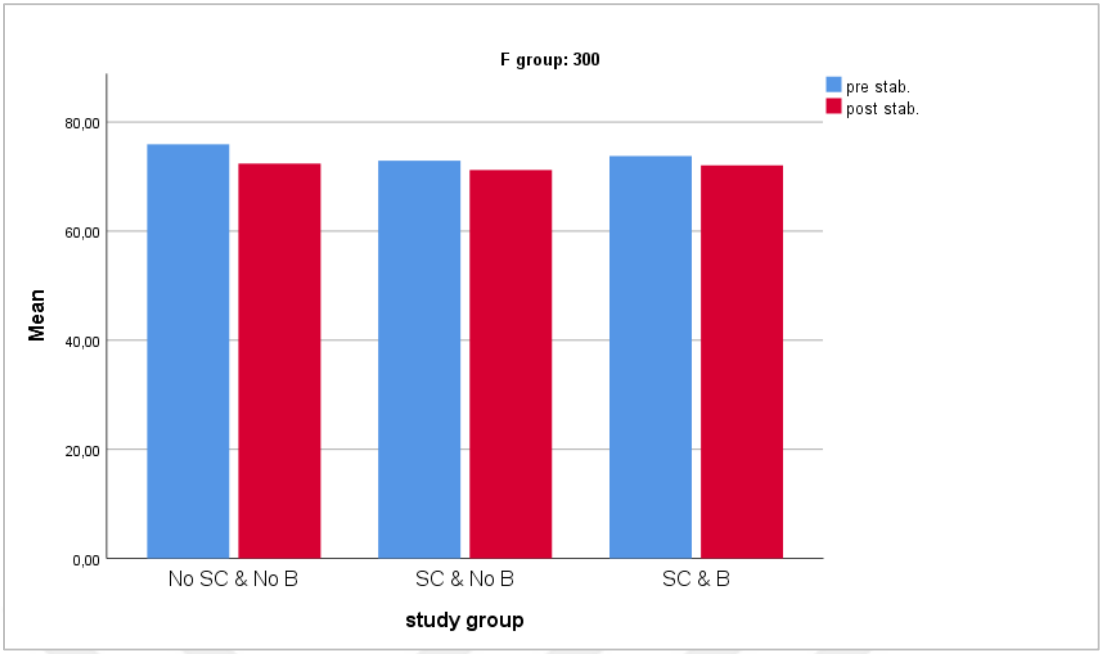


**Figure 4.3**: Graphic representation of the pre- and post- stability ISQ values while applied force was 300 cN.

All 77 miniscrews could be inserted with high primary stability (mean ISQ values: 74.73 and 72.80 post-operatively).

**Table 4.6:** Mean ISQ values for reaching a standardization.

| | n (MSI) | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| ISQ in T1 | 77 | 65.25 | 89.50 | 74.7390 | ±4.62705 |
| ISQ in T2 | 77 | 65.25 | 84.00 | 72.8019 | ±4.34857 |

Table 4.7 and Figure 4.4 shows the comparisons of BV/TV at the end of study (T2) in 3 main groups (A stands for No SC, B stands for SC, C stands for SC+Boron). In B and C groups, bone volume to tissue volume ratio has been increased in comparison to control groups. Although this change is not considered statistically significant (p = 0.053).

**Table 4.7:** Descriptive statistics and comparisons of BV/TV at the end of study in 3 main groups.

| Study Groups | n (MSI) | Min | Max | Mean | Std. Deviation | p* |
|---|---|---|---|---|---|---|
| A | 8 | 0.38 | 0.57 | 0.47 | ±0.06 | |
| B | 6 | 0.49 | 0.69 | 0.60 | ±0.08 | 0.053 |
| C | 6 | 0.41 | 0.65 | 0.50 | ±0.09 | |

*p values were calculated from Kruskal-Wallis test, $p < 0.05$

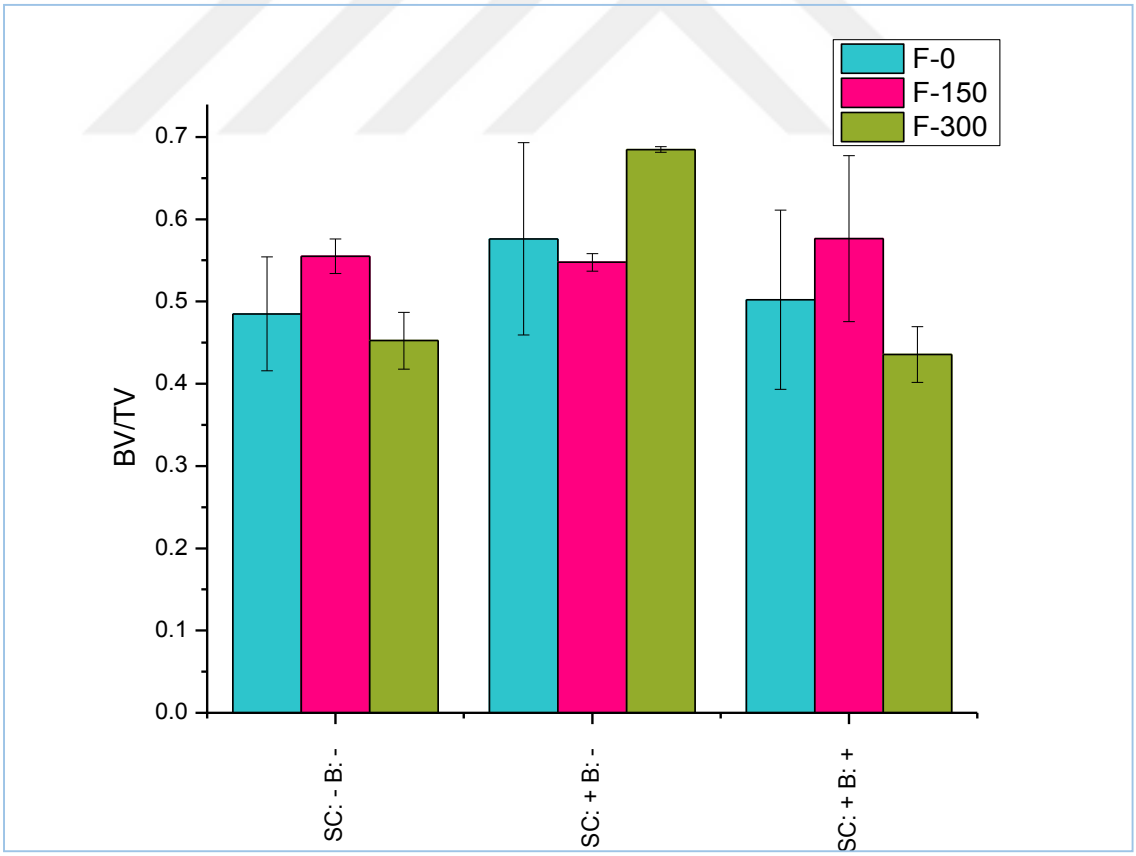


**Figure 4.4**: Graphic representation of bone volume to tissue volume ratio

Table 4.8 and Figure 4.5 shows the comparisons of BS/BV at the end of study (T2) in 3 main groups. In B and C groups, bone surface to bone volume ratio has been decreased in comparison to control groups. Although this change is not considered statistically significant (p = 0.061).

**Table 4.8:** Descriptive statistics and comparisons of BS/BV

| Study Groups | n (MSI) | Min | Max | Mean | Std. Deviation | p* |
|---|---|---|---|---|---|---|
| A | 8 | 2.98 | 7.60 | 4.60 | ±1.65 | |
| B | 6 | 1.92 | 4.56 | 2.75 | ±1.00 | 0.061 |
| C | 6 | 1.95 | 5.34 | 3.67 | ±1.48 | |

*p values were calculated from Kruskal-Wallis test, p < 0.05

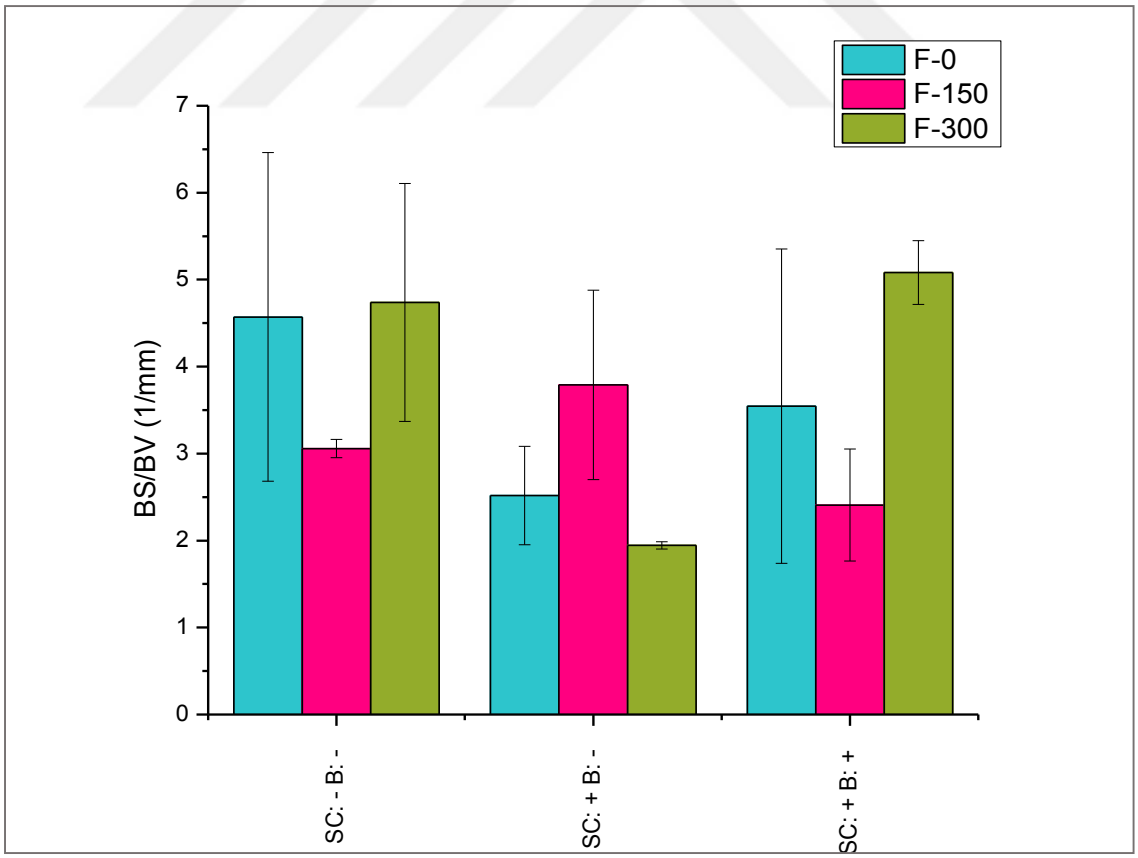


**Figure 4.5**: Graphic representation of bone surface to bone volume ratio

Table 4.9 and figure 4.6 shows the comparisons of trabecular thickness at the end of study in 3 main groups. In B and C groups, trabecular thickness has been increased in comparison to control groups. Although this change is not considered statistically significant (p = 0.061).

**Table 4.9:** Descriptive statistics and comparisons of trabecular thickness

| Study Groups | n (MSI) | Min | Max | Mean | Std. Deviation | P* |
|---|---|---|---|---|---|---|
| A | 8 | 0.44 | 1.04 | 0.48 | ±0.15 | |
| B | 6 | 1.92 | 4.56 | 0.79 | ±0.24 | 0.061 |
| C | 6 | 0.37 | 1.02 | 0.63 | ±0.27 | |

*p values were calculated from Kruskal-Wallis test, p < 0.05

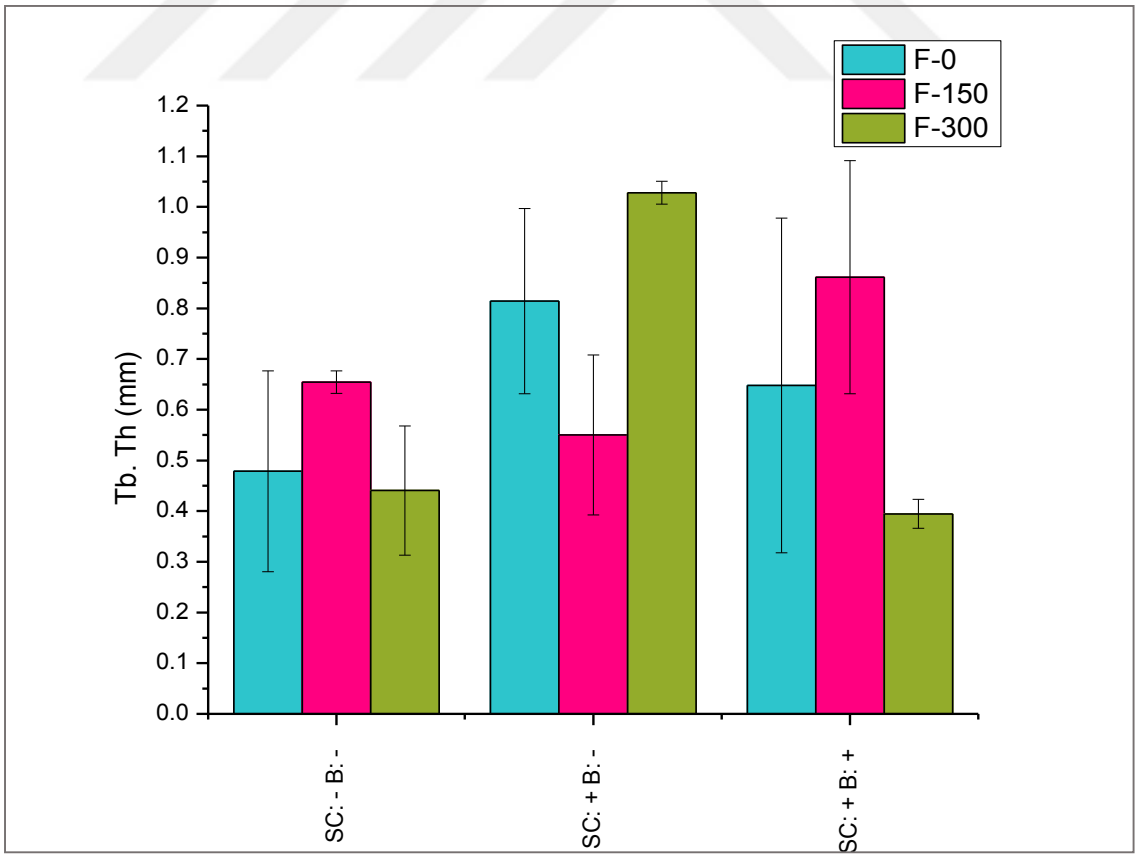


**Figure 4.6**: Graphic representation of trabecular thickness

Table 4.10 and Figure 4.7 shows the comparisons of trabecular number at the end of study in 3 main groups. In B and C groups, trabecular number has been decreased in comparison to control groups. Although this change is not considered statistically significant (p = 0.099).

**Table 4.10:** Descriptive statistics and comparisons of trabecular number

| Study Groups | n (MSI) | Min | Max | Mean | Std. Deviation | p* |
|---|---|---|---|---|---|---|
| A | 8 | 0.55 | 1.46 | 1.05 | ±0.23 | |
| B | 6 | 0.66 | 1.23 | 0.80 | ±0.21 | 0.099 |
| C | 6 | 0.63 | 1.11 | 0.87 | ±0.22 | |

*p values were calculated from Kruskal-Wallis test, p < 0.05

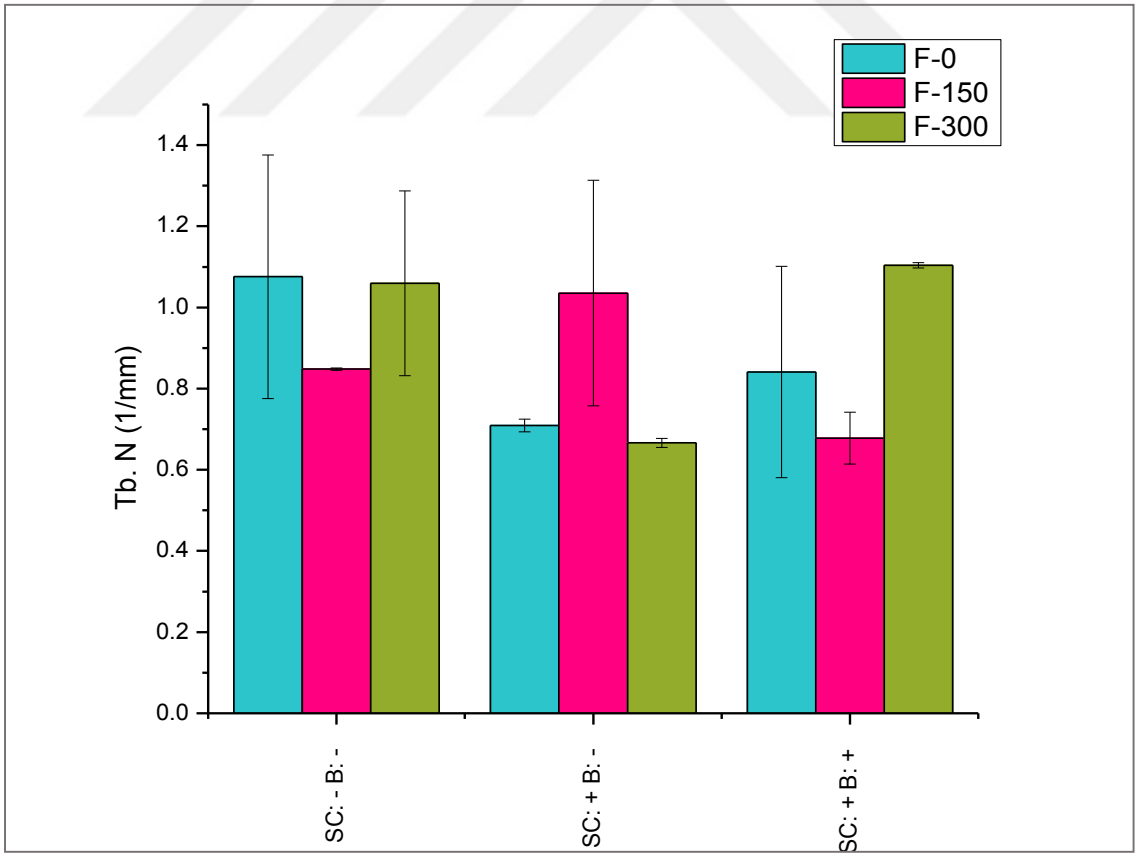


**Figure 4.7**: Graphic representation of trabecular number

Table 4.11 and Figure 4.8 shows the comparisons of trabecular separation at the end of study in 3 main groups. Trabecular separation has been increased only in C groups in comparison to control groups. Although this change is not considered statistically significant (p = 0.159).

**Table 4.11:** Descriptive statistics and comparisons of trabecular separation

| Study Groups | n (MSI) | Min | Max | Mean | Std. Deviation | p* |
|---|---|---|---|---|---|---|
| A | 8 | 0.42 | 0.58 | 0.50 | ±0.05 | |
| B | 6 | 0.37 | 0.70 | 0.50 | ±0.10 | 0.159 |
| C | 6 | 0.49 | 0.68 | 0.57 | ±0.07 | |

*p values were calculated from Kruskal-Wallis test, p < 0.05

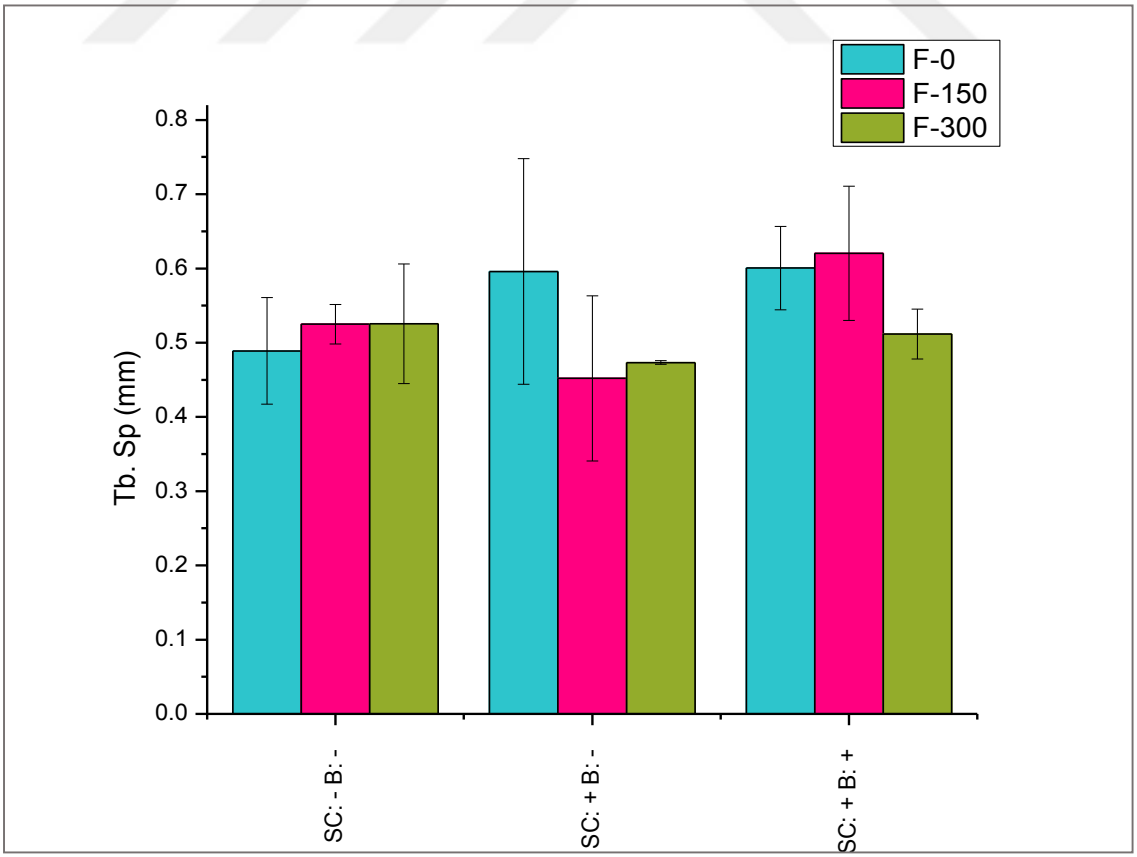

**Figure 4.8**: Graphic representation of trabecular separation

Table 4.12 and Figure 4.9 shows the comparisons of closed porosity at the end of study in 3 main groups. In B and C groups, closed porosity has been decreased in comparison to control groups. Although this change is not considered statistically significant (p = 0.097).

**Table 4.12:** Descriptive statistics and comparisons of closed porosity

| Study Groups | n (MSI) | Min | Max | Mean | Std. Deviation | p* |
|---|---|---|---|---|---|---|
| A | 8 | 0.16 | 0.63 | 0.39 | ±0.20 | |
| B | 6 | 0.09 | 0.49 | 0.23 | ±0.13 | 0.097 |
| C | 6 | 0.09 | 0.25 | 0.17 | ±0.06 | |

*p values were calculated from Kruskal-Wallis test, p < 0.05

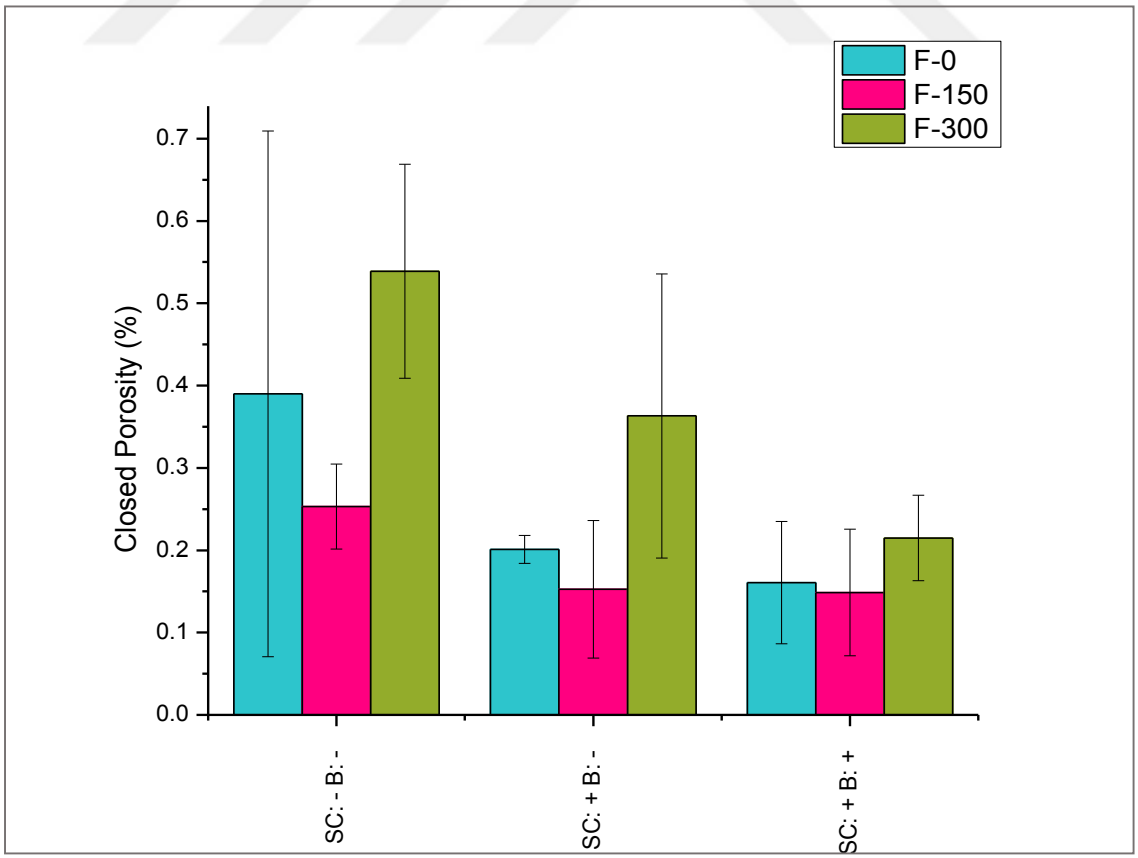

**Figure 4.9**: Graphic representation of closed porosity

Table 4.13 and Figure 4.10 shows the comparisons of open porosity at the end of study in 3 main groups. Only in C groups, open porosity has been increased in comparison to control groups. Although this change is not considered statistically significant (p = 0.098).

**Table 4.13:** Descriptive statistics and comparisons of open porosity

| Study Groups | n (MSI) | Min | Max | Mean | Std. Deviation | p* |
|---|---|---|---|---|---|---|
| A | 8 | 42.96 | 56.97 | 50.03 | ±5.79 | |
| B | 6 | 30.81 | 50.53 | 39.51 | ±8.48 | 0.098 |
| C | 6 | 34.97 | 58.75 | 49.41 | ±9.32 | |

*p values were calculated from Kruskal-Wallis test, p < 0.05

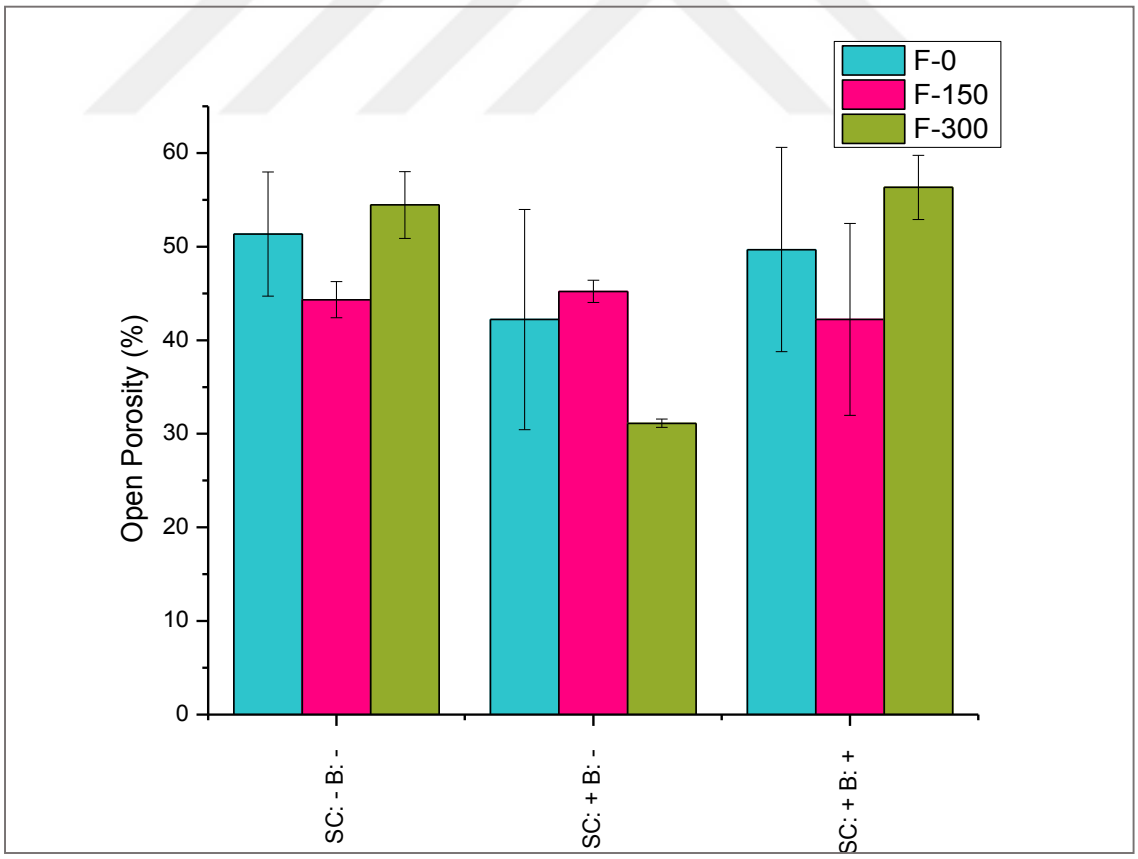


**Figure 4.10**: Graphic representation of open porosity

## 5. DISCUSSION and CONCLUSION

According to Serra et al. (61) reaching primary stability in the placement time is fundamental for the maintenance of the mini-implant, but it does not guarantee the success.

For promoting cell bio-stimulation and enhancing the regenerative capacity of bone around mini implant in orthodontics, some therapeutic alternatives are being introduced.

Stem cell therapy is considered a novel alternative for the treatment of clinical conditions that requires tissue regeneration in Orthopedics and Periodontology. In this blinded controlled experimental animal study, the effects of adipose derived stem cells on stability enhancement and bone regeneration around orthodontic mini screw implants has been investigated. To our knowledge, this is the first study in orthodontics that shows the effects of stem cell therapy on stability and bone formation of mini screw implants (3, 5, 11, 12). Bio-stimulating methods hold potential for enhancing the stability of MSIs. Among the most powerful pharmacologic agents available are the bisphosphonates, which limits bone resorption by inhibiting osteoclastic activity (13- 14).

Cuarian et al. (3) demonstrated that one small locally delivered dose of Zoledronate maintained the stability of MSIs over time, primarily because of greater amounts of trabecular bone surrounding the MSIs in dogs.

Photobiomodulation therapy (LPT) to enhance mini implant stability is the other method for enhancing bone regeneration in cell level; Uysal et al. (5) demonstrated that orthodontic miniscrews that irradiated by LED photobiomodulation had greater stability than the non-irradiated control miniscrews; at 21 days post-screw placement, implants had greater stability in cortical tibia bone of rabbit,and they suggested that LPT might have a favorable effect on healing and attachment of titanium orthodontic miniscrews.

Recently Bayani et al. (10) evaluated the effect of platelet-released growth factor (PRGF) and immediate orthodontic forces on the removal torque of miniscrews in dogs, following 12 weeks they concluded that applying PRGF with miniscrews increased their stability.

**Dosage and delivery of SC**

The number of stem cells applied in each of our experimental mini implant pilot holes before insertion of mini implants in was as 250.000 cells in 50μL, which is 10 times more than the amount of cells described by Zheng et al, applied in to the prefabricated holes in dental implants. The fact that in their study they had an area to settle the stem cells and mix it with hydroxyapatite/ tricalcium phosphate a carrier powder may caused them to achieve some significant result from a histologic standpoint (125). The number of stem cells applied in each pilot holes before insertion of mini implants in our study is the half of the amount of stem cell of a study by Ekizer et al. (100) that used a single injection of bone marrow derived stem cell in each midpalatal suture of rats 24 hours after expansion of the suture to increase the bone formation process.

In our study, stem cells was injected into the pilot drill hole. Although this method was previously implemented in literature, it seems that there is a need to develop a scaffold like medium to hold the stem cells more efficiently. Some authors stated their concerns about surface characteristics of the miniscrew, such as its hydrophilic/hydrophobic affinity, which can affect its wettability while injecting growth factors. (16).

**Animal Model**

The animal study model seems to be a useful method to investigate a bioactive therapy on bone tissues. Different animal models have been studied for investigating mini implant stability in the literature, such as dogs, rabbits and rats. In order to be able to compare the results more accurately, rabbit was chosen because this animal is most frequently used when the study design is in-vivo. However, any results from an experimental animal model should be not directly extrapolated to humans.

In a routine clinical procedure for implant placement, mini implants are usually placed in the attached gingiva without any flaps. In this situation the head and auxiliaries used around the implant increases the risk of infection when compared to closed flap technique which was used in our study. Although rabbits tend to bite on the stiches and this may add further complications. Using gauzes to cover the surgical site minimized any adverse effect of closed flap technique in rabbits (3).

**Method of Measurement for Stability**

In our study the stability of the mini implants with RFA was measured, which is considered superior for clinical use to torque measurement device.

According to Cochran (64), the gold standard for primary stability measurements would be the histologic and histomorphometric analyses of primary bone contact. However, neither histology nor histomorphometry is performed routinely in the clinical situation for large numbers of implants.

As the torque is the one time, static measurement at placement of the implant and cannot be the repeated later on in a noninvasive way. It measures the rotational friction between the implant surface and the bone and not the lateral stability of implant. RFA only vibrates the SmartPeg, and not affecting the implant itself (58).

Periotests (Medzintechnik Gulden e K, Modautal, Germany) device is designed for natural teeth that are attached to the bone with a periodontium. An implant is attached directly to the bone without periodontium. RFA uses a more sensitive scale with a non-contact, non-invasive techniques which is totally objective and repeatable.

Currently RFA is regarded as the gold standard for clinical stability measurement of dental implants. Therefore, it would be desirable to use it to evaluate orthodontic mini-implants as well (65).

Although for implementing RFA analysis in mini implant a major obstacle is the connection between the mini-implant and the SmartPeg. Because of the sensitivity of this measurement technique, a stable and reproducible connection must be ensured to make it work. Most dental implant systems have an inner screw thread so the RFA system is based on a screw coupling. The first pilot studies regarding RFA for mini-implants used adhesive fixation of a magnet to the mini-implant's head. The results suggested that RFA might work for mini-implants (58). Recent studies questioned the reproducibility or stability of this kind of attachment; therefore, they started to modify the head of mini-implants into an external screw head. This can be useful for stability measurement in animal studies if it is made accurately, but for clinical use this would prevent attaching any kind of mechanics for tooth movement. In our study the mini implant were fabricated in order to have a custom built in thread in head to make it possible to be used in clinical setting (5). This approach was used by Nienkemper et al. (36) previously. It is made for fixation of prefabricated abutments to create different kind of mechanics. This kind of built in head may be used for attachment of auxiliaries in clinical use.

Another point in using RFA for mini-implants is the reduced size compared with dental implants. Implant size significantly affects the level of resonance frequency. Regarding the operating mode of RFA, it has to be proven whether the resonance frequency of mini-implants in bone fits the range of frequency emitted by the Osstell ISQ. Otherwise the results would not be sensitive or significant. So the validation of RFA for mini-implants should be performed before using it as measuring method in further studies or clinical routine (36).

Because of the significant differences in length and diameter between dental implants and orthodontic mini-implants, there is still no standard range set for an orthodontic mini-implant. Nienkemper et al. (36) showed that RFA can be used as measurement method for mini-implant stability in clinic; and this method might be used routinely to assess mini-implant stability throughout the course of treatment in the future.

All of the MSIs in our study remained stable during the follow up period. It was determined that all of MSIs was inserted with high primary stability in control, and experimental groups. This is in accordance with the data collected by Uysal et al. (5), assessing light-emitting diode photobiomodulation therapy on mini implants with the same follow up duration. But the range of median ISQ levels in our study is considerably higher values (69.75-76.87) in comparison to their study (49.25-55.00). Although in their study ISQ values of all miniscrews were found higher in experimental group at the end of observation period in comparison to the control. In our study study ISQ values of all miniscrews showed minor decrease in follow up, although this decrease is not considered statistically significant.

Ure et al. (72) pointed to the issue that screwing the Smartpeg into the head of the MSI and unscrewing it after the recordings may have contributed to the high failure rate, because all of the failures in their study occurred while attempting to unscrew the Smartpeg, however our results demonstrated that this issue had no influence in the study results.

**Osseointegration**

The osseointegration phenomenon for miniscews used in orthodontics has been histologically confirmed in previous studies (39).

Orthodontic mini-implants are mainly used for primary loading and not for the actual osseointegration process, as is suggested for conventional dental implants or orthodontic palatal implants (40).

According to Woods et al. (41) only small amounts of ossteointegration may be required for stability because orthodontic forces are substantially less than the occlusal loads placed on traditional endosseous implants. Moreover, masticatory forces produce dynamic, or intermittent, loads with variable forces, as compared with constant loads produced by orthodontic forces. Therefore, miniscrews can be loaded immediately before osseointegration because stability is supported by the mechanical retention between the implant and the bone tissue. However; magnitude, direction, and pattern of orthodontic force could influence stability (5). The dilemma regarding miniscrews is that while they should have long-term stability for clinical applications, yet it should be easy to remove them after treatment (54). This concern was previously stated by Ohmea et al. (42), that orthodontic miniscrews can achieve partial osseointegration after 3 weeks, increasing the difficulty of their removal. In this regard Melsen et al. (30) concludes that the mini- screw typically can be removed without complications a few days after the first attempt of removal.

**Force**

No force, 150 cN and 300 cN has been applied to MSIs in the present study. Immediate loading of 150 cN and 300 cN versus no loading did not make any difference in the ISQ value changes during study period from the statistical standpoint.

Most clinical reports suggest that TADs are stable with applied forces ranging from 50 cN to 450 cN (43, 44).

Deguchi et al. (45) suggested to provide a healing period before loading, in order to increase the secondary stability.

On the contrary; Melsen et al. (46) stated that immediate loading seems to have a positive effect on bone, increasing the cellular turnover and density in the areas adjacent to loaded implants in comparison with implants with no force applied, suggesting that orthodontic loading may have a protective effect. This finding is in agreement with Manni et al. (88) and Kuroda et al. (93) that found immediate loading seemed to have a positive and significant infuence on the success rate.

Almost the same as Uysal et al. (5) at the end of follow-up period, we observed no significant difference between the ISQ values of loaded and unloaded implants. This finding was not in accordance with Woods et al. (41) that indicate immediate loading could be beneficial because in their study mobility was observed only in unloaded control miniscrews.

In 2010, Crismani et al. (47) in a review of published clinical trials, came in to the conclusion that immediate or early loading of miniscrews is possible, since longer healing periods did not provide additional stability at forces of up to 200 cN. But Miyawaki et al. (23) concluded that immediate loading with less than 2 N (203.9 g) was not detrimental to MSI success. Cornelis et al. (48) reported that MSIs have been successfully loaded immediately with 25 to 500 g (0.245- 4.903 N) of tensile force perpendicular to the implant in various animal experiments. Chen et al. (49) suggested that immediate loading of less than 200 g will be successful. Therefor in this study 150 cN was considered as a normal force that is normally used in clinical situations. The important factor and matter of concern in some situations is the maximum load that a mini screw can withstand without loosing its stability. Roberts et al. (50) stated that forces between 1 and 3 N did not affect the implant's stability.

**Maximum Force**

In this study, 300 cN was considered as the maximum load and is achieved by using shorter coil spring and measuring with gauge.

Büchter et al. (51) showed that tip forces higher than 9 N resulted in a high risk for osseointegration loss for dental implants.

Mortensen et al. (52) compared the stability of 3- and 6-mm long miniscrew implants loaded with orthopedic force levels (600 gr and 900 gr). MSIs immediately loaded with 600 or 900 g of force experienced significant linear displacements during their study.

Isidor (53) added that high forces tend to damage the interface integration.

Woods et al. (41) in a study assessing the effect of force, timing, and location on bone-to-implant contact of miniscrew implants, found that the maximum load did not have a negative effect on the amount of bone implant contact around the immediately loaded MSI.

As stated by Owens et al. (54) the lack of effect on bone implant contact indicates that the maximum load limit is likely to be above 50 g and they suggested that further studies will be needed in order to determine the maximum force level permissible for the loading of MSIs.

**Success Rate**

Our ability to control all aspects this study probably accounted for a 100% success rate. None of the 78 MSIs failed, and all remained clinically stable during the 3 experimental weeks. Systematic reviews indicate that MSIs placed in humans are successful 80% to 84% of the time. Animal studies consistently report higher success rates (83.6%-100%), that was applicable to our study (14, 15, 18, 47). In our study all of the mini screws had a high initial mechanical stability, and any intervention to enhance osseointegration such as stem cell therapy with or without boron could not ass stability in a significant way. It may be assumed that these kind of auxiliary therapies come to help in less ideal situations. In clinics there are some situations that its is not in favor of mini implant stability.

The data from 2 other studies that implemented cell biomodulation therapy for enhancing mini implant stability in the literature can not extrapolated to our study, because in both of these studies the factor of force is completely ignored while assessing the mini implant stability (3, 6).

**Force in Cell Biology**

Our cortical bone thickness values were comparable with previously reported values for rabbits, in this study to standardize all the mini implants were inserted at the similar distance from the head of the tibia (55). A systematic review and meta-analysis on the influence of the cortical thickness on primary stability of miniscrews, revealed a positive association between MI primary stability and cortical thickness of the receptor site; with the consideration of the fact that the available trials are not well-designed.

Mechanical influences on biological processes, known as mechanobiology, significantly affect all phases of bone formation. Mechanical signaling at the cellular level may modulate molecular changes in cytoskeleton, integrins and ion channel activities with consequences to the differentiation and gene expression of cells involved

in the healing process, thus it may be concluded that adding force in a study design may have beneficial effects in healing process (119).

**Follow up Duration**

The follow up period in the present study was 3 weeks which is in accordance with the design of the study by Uysal et al. (5).

Ure et al. (72) argued that the point of transition from primary to secondary stability appears to occur at approximately 3 weeks, which is comparable to the point of transition identified for dental implants. Although our follow up is also 3 weeks, it should be kept in mind that the bone turnover rate is faster in animals and it differs between animal species and this finding may not be extrapolated to all of the studies.

Decreases in ISQ values during the first 3 to 4 weeks have been previously reported after dental implant placement. Stability might be expected to start decreasing within the first week, when osteoclasts and mesenchymal cells, which appear by day four, begin removing bone damaged during MSI placement. Strategies to reduce trauma to bone during insertion or ways to accelerate healing should produce greater MSI stability (80).

**Pilot Holes**

Due to the brittleness of the rabbit bone, using pilot holes prior to implant placement in rabbit tibia was very important in the present study. Ure et al. (72) stated that when measuring mini implant stability with RFA, Whether or not the MSIs were placed with or without pilot holes appeared to have no appreciable effect on changes of ISQ values. It is possible that small differences actually exist that RFA may not be sensitive enough to detect. A more plausible explanation relates to the stiffness of the bone, which is one of the factors that determine the resonance frequency of an implant. Because the stiffness of the bone is a function of its physical composition, it might be expected to remain the same whether or not a pilot hole is placed. It is also possible that the placement of a pilot hole causes as much trauma to the bone as does the placement of a MSI without a pilot hole.

**µCT**

µCT provides a more powerful method for evaluating bone-implant contact, perhaps because it summarizes all of the bone with the capacity to detect subtle bone-density changes surrounding the MSI, rather than being restricted to a single histomorphometric slice (4).

In the present study positive contribution of SC therapy to bone formation could not be confirmed statistically significant in a primary µCT analysis. In 2010, Lee and Baek (90) investigated the effects of diameter and shape of MSIs with regard to micro damage in the cortical of rabbit bone during MSI placement. As a part of study they used Micro-CT to measure cortical bone thickness precisely without damaging bone and MSIs. They found that the thickness of cortical bone adjacent to the MSIs in the rabbits it their study was about 0.7 mm and slightly less than that of the human maxilla: 0.85 to 1.03 mm at the labial and buccal areas.

In this study the layer adjacent to the implant is has been excluded because of possible metallic artifacts. Ikeda et al.in 2011, evaluated the effects of surface modifications of MSIs and force application on bone surrounding MSIs in dogs with, and they evaluated bone volume to total volume ratios of cortical and noncortical bone regions from the entire MSI surface using µCT analysis to compare MSIs with sandblasted, large-grit, and acid-etched (SLA) surfaces. In their study the same method of exclusion was implemented.

Although µCT analysis gives a superior information about bone quality three dimensionally, to ensure that the applied stem cells have participated in to the new bone structure, labeling the stem cells prior to application into the bone with nuclear counterstain, DAPI (49,6- diamidino-2-phenylindole) and histomprphometric evaluations may confirm this concern (100).

**SC**

The cell origin may have some affects on optimal differentiation and regeneration, Hans et al. in a study about bone regeneration effects of BMSC and ASC collected stem cells from these two sources and compared amounts of bone regeneration from both groups of cells. They have concluded that adipose-derived stem cells differentiate directly into osteoblasts less often than do bone marrow-derived stem cells. However, the total amount of regenerated bone is almost the same because of the effect

of indirect bone regeneration. As adipose-derived stem cells are easily accessible and have the potential to abundantly proliferate into mesenchymal cells, they could be an effective bone regeneration material. Thus, although the direct bone regeneration effect of adipose-derived stem cells is relatively weak, the indirect bone regeneration effects of bone development protein and various growth factors can effectively compensate for that weakness (112).

According to Salehi-Nik et al. (113), The buccal fat pad is a specialized adipose tissue that is easy to harvest and contains a rich blood supply, and its harvesting causes low complications for patients. This fact facilitates the use of ACS in clinical setups in comparison to other sources.

Fracon et al. (154), stated that administration of nonsteroidal anti-inflammatory drug (NSAID), by the veterinarian to animals after surgical intervention to control pain, can cause bone formation inhibition, because of prostaglandin formation inhibition. In our study we paid attention to this issue and systemic administration of NSAID was only took place when the need for that was affirmed by veterinary doctor.

**Boron**

Boron derivatives (even boric acid) had been first investigated in 2013 for the molecular mechanism of stem cell differentiation and bone and teeth growth stimulation by Tasli et al. (134) They have shown that sodium pentaborate pentahydrate is less cytotoxic to mammalian cells compared to boric acid. Therefore, sodium pentaborate pentahydrate is a good alternative as boron source in bone and tooth regeneration studies. They have shown that sodium pentaborate pentahydrate (NaB) can increase osteogenic and odontogenic differentiation of human tooth germ stem cells (hTGSCs) in vitro.

Although in our study using boron prior to implant placement did not add any significant changes in ISQ value or bone quality, the ultimate importance of NaB in stem cell performance, specially the osteogenic differentiation of MSCs has been confirmed in recent years (137-140). Uysal et al. (141) found that Boron has a positive effect on the early phase of bone regeneration of the mid- palatal suture in response to expansion and may be beneficial in routine maxillary expansion procedures. In our study although some changes in Boron group has been observed, the changes were not statistically significant.

The future advancement of tissue engineering in craniofacial hard tissue reconstruction will continuously focus on three essential components: mesenchymal stem cells, scaffolds and growth factors. While animal studies and preclinical models further contribute to the clinical practice of regeneration, several major challenges need to be addressed: Safety issues hamper the advancement of SC-based innovative therapies and raise the need for novel standards to adequately address and rule out concerns, considering the permanent nature of SC treatments. Many biological aspects concerning dose, time and method of administration are still to be elucidated (123).

**Conclusion**

Within the limits of the present randomized controlled experimental animal study;

- At the baseline, it was determined that all miniscrews could be inserted with high primary stability and accepted that initial stability of all was similar in experimental and control groups.
- ASC were isolated from the fat tissue of rabbit and were characterized and underwent osteogenic induction. They were implemented prior to mini implant insertion. Positive contribution to bone formation could not be confirmed statistically significant in a primary μCT analysis.
- All the mini implants remained stable throughout the study as assessed by RFA, although a significant difference in ISQ values could not be observed between main groups.
- Immediate loading of 150 cN and 300 cN versus no loading did not make any difference in the ISQ value changes during study period from the statistical standpoint.
- Non of the mini implants in our study showed any sign of inflammation, besides using Dermobor prior to implant placement did not add any significant changes in ISQ value or bone quality.

The null hypothesis was accepted that there is no significant difference between the orthodontic mini screw stability of Stem cell-treated; with or without Boron versus non-Stem cell treated rabbits under different force levels.

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# 7. APPENDICES

## 7.1. Ethical Approval

Permission was obtained from the Yeditepe University; (protocol 535) Ethics Committee of Experimental Animals after the Research Scientific Committee at the same institution had approved the experimental protocol. The rabbit purchase, selection, and management, and the experimental procedures were carried out in the Yeditepe University Experimental and Clinical Research Center. The experiments were performed in accordance with the Animal Welfare Act of 20 December 1974, No. 73, Chapter VI Sections 20–22 and the Regulation on Animal Experimentation of 15 January International Standard ISO 10993-6, 1994.

YEDİTEPE ÜNİVERSİTESİ

**T.C. YEDİTEPE ÜNİVERSİTESİ, DENEY HAYVANLARI ETİK KURULU**
**(YÜDHEK)**

**ETİK KURUL KARARI**

| Toplantı Tarihi | Karar No | İlgi | Proje Yürütücüsü |
|---|---|---|---|
| 24.05.2016 | 535 | 4.05.2016 | Nasim Mesgarzadeh |

**'Effects of mesenchymal stem cells on bone formation and stability of orthodontic mini implants'** adlı bilimsel çalışma etik kurulumuzda görüşülmüş olup, çalışmanın etik kurallara uygun olduğuna oy birliğiyle karar verilmiştir.

| | | |
|---|---|---|
| **Etik Onay Geçerlilik Süresi**: 1 Yıl | **Hayvan Türü ve cinsiyeti:** Rabbit ♂ | **Hayvan Sayısı:** 17 |

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

PERSONAL INFORMATION

Nasim Mesgarzadeh

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EDUCATION

- **Residency and PhD Degree Candidate in Orthodontics and Dentofacial Orthopedics**, Yeditepe University, Faculty of Dentistry and Dental Hospital, Sep 2012 - April 2018

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  Supervisor: Prof. Dr. Didem Nalbantgil
- **Orthodontics Preceptorship Certificate**, UCLA Dental school, Los Angeles, USA; Jun 2011 - Dec 2011
- **Advanced Implantology Preceptorship Certificate**, UCLA Dental school, Los Angeles, USA; Mar - Jun 2011
- **DDS** Degree, Azad University, Tehran Faculty of Dentistry, Tehran, Iran; Sep 2003 - Feb 2010, GPA evaluated by ECE: 3,45

  DDS Thesis Subject: Evaluation of Nickel and Chromium ions in saliva of the orthodontic patients with fixed appliances_ 1-year prospective cohort study

  Supervisor: Prof. Dr. Fariborz Amini

LICENSURE

Permanent license of Dentistry, Iran, 2010

MEMBERSHIPS

- American Association of Orthodontics and Dentofacial Orthopedics
- World Federation of Orthodontists
- European Association of Orthodontists
- Iranian society of Orthodontists
- Turkish Orthodontics Society
- Turkish Cleft lip and palate society
- Iranian Medical Council
- Iranian Dental Association

PUBLICATIONS

- Yilmaz RB, Cakan DG, Mesgarzadeh M. Prevalence and management of natal/neonatal teeth in cleft lip and palate patients. Eur J Dent. 2016 Jan-Mar;10(1):54-8 PIMD: 27011740
- Amini F, Rakhshan V, Mesgarzadeh N. Effects of long-term orthodontic treatment on salivary Nickel and Chromium levels: a 1-year prospective cohort study. Biol Trace Elem. 2012 Dec;150(1-3):15-20 PIMD: 22644664

RESEARCH EXPERIENCES

- Animal use in experimental research certificate course, **International Certificate**, Istanbul, Turkey, Jan 2015
- Extraction of mesenchymal stem cells for biological use, Department of Genetics and Bioengineering, Yeditepe University, Istanbul, Turkey
- SkyScan Micro CT workshop, Department of Nano technology, Sabanci University, Istanbul, Turkey
- The effect of periodontal therapy on C-reactive protein and Interleukins in serum and GCF_ a systematic review and meta analysis , project under supervision of Head of Dental research and Science, Azad University, Tehran, Iran, Sep 2009 - Jan 2011

LANGUAGE SKILLS

Persian (native), English (proficient), Turkish (proficient), French (fluent)

PRESENTATIONS

- **Revolutionary stem cell therapy for bone regeneration; current status and potential applications in orthodontics**

  13th International Congress of Iranian Association of Orthodontists, Iran, Feb 2017; **Oral Lecture**
- **Interdisciplinary management of an orthodontic patient: Esthetic outcomes and Dolphin visual treatment objective (VTO) accuracy assessment**

  XV international Congress of Turkish orthodontics Society, Turkey, 2016; poster presentation
- **Bone supported molar distalization appliance delivering double side force, a case report**

  8th International Orthodontic Congress of World Federation of Orthodontics, London, 2015; poster presentation
- **Prevalence of natal and neonatal teeth in infants with cleft lip and palate**

  8th International Orthodontic Congress of World Federation of Orthodontics, London, 2015; poster presentation
- **Fixed mini implant assisted orthodontic/orthopedic appliance for cross bite correction and mandibular functional therapy**

  XIV International Congress of Turkish Orthodontists Society, 2015; poster presentation
- **Rapid maxillary expansion in young adult using bone borne and tissue borne expander**

  XIV International Congress of Turkish Orthodontics Society, 2015; poster presentation
- **Non surgical orthodontic treatment of a skeletal class lll malocclusion, a multidisciplinary case report**

  XIV International Congress of Turkish Orthodontics Society, 2015; poster presentation
- **The orthodontic and orthognatic treatment of a skeletally class lll patient, a multidisciplinary case report**

  XIV International Congress of Turkish Orthodontics Society, 2015; poster presentation
- **Root resorption during and after orthodontic treatment**

5th Congress of Iranian association of General Dentists, Tehran, 2011; Poster Presentation

- **Xerostomia**
  4th National Congress of Oral Disease, Tehran, 2010; **Oral lecture**
- **Pit and fissure sealant therapy in children and adolescents**
  4th Congress of Iranian association of General Dentists, Tehran, 2010; Poster presentation
- **Fluoride releasing in Dental Materials**
  10th Congress of Iranian Academy of Restorative Dentistry, Tehran, 2010; Poster presentation

CONFERENCES AND CONTINUING EDUCATION COURSES

- Biomechanics and esthetics based orthodontic treatment strategies, Dr **Ravindra Nanda**, Istanbul Feb 2018
- **Invisalign** Certification Course, Dr Ahmad Hagar, Istanbul, Nov-Dec 2017
- Clinical technologies to improve efficiency and efficacy, Dr **John R. Bob Smith**, Istanbul, May 2016
- **American Association of Orthodontists winter** conference, Palm springs, Feb 2016
- 3M **Incognito** course, Dr Roberto Stradi, Istanbul, Oct 2015
- Orthognatic surgery preparation, Istanbul, Dr **Korkmaz Sayinsu**, Jun 2015
- **American Association of Orthodontists Annual** session, SF, May 2015
- **Sleep apnea**, Acibadem Hospital, Istanbul, Dec 2014
- Carriere motion appliance in self ligating system, Dr. **Luis Carriere**, Istanbul, Dec 2014
- **Maxillofacial surgery** and Orthodontics, Yeniyuzyil University,Istanbul, Dec 2014
- Comprehensive **Damon** System course, Istanbul, Nov 2013
- Diagnosis, treatment planning and treatment mechanics, Dr. **Richard P. Mc Laughlin**, May 2013
- **International Congress of Turkish Orthodontists Society**, 2012 to 2016
- **3D virtual** versus traditional orthognatic treatment planning, Los Angeles Center for oral and maxillofacial surgery (LACOMS), Nov 2011

- **Cleft palate annual Symposium**, Los Angeles, Oct 2011
- **Dolphins CAD/CAM**, 3D and orthodontics, Los Angeles, Sep 2011
- Advanced implant therapy week, Dr. **Sacha A. Jovanovic**, UCLA, Sep 2011
- **Medical emergencies** in dental office, Azad dental school, Tehran, Dec 2009
- Advanced **Pharmacology** course for Dentists, Tehran, Nov 2006