T. C.
ERCIYES UNIVERSITY
FACULTY OF DENTISTRY
Department of Oral and Maxillofacial Surgery

THE EFFECTS OF PTH (1-34) AND SERM (RALOXYPHENE) ALONE, IN SEQUENTIAL OR IN COMBINATION APPROACHES ON OSSEOINTEGRATION OF TITANIUM IMPLANTS IN OSTEOPOROTIC RABBIT MODEL

PREPARED BY

Firas MOHSEN

SUPERVISOR

Asst. Prof. Dr. Ahmet Emin DEMİRBAŞ

DOCTORAL THESIS

2020

KAYSERİ

T. C.
ERCIYES UNIVERSITY
FACULTY OF DENTISTRY
Department of Oral and Maxillofacial Surgery

THE EFFECTS OF PTH (1-34) AND SERM (RALOXYPHENE) ALONE, IN SEQUENTIAL OR IN COMBINATION APPROACHES ON OSSEOINTEGRATION OF TITANIUM İMPLANTS IN OSTEOPOROTIC RABBIT MODEL

(Ph.D. Thesis)

PREPARED BY

Firas MOHSEN

SUPERVISOR

Asst. Prof. Dr. Ahmet Emin DEMİRBAŞ

This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) with project number 217S387

DOCTORAL THESIS

2020

KAYSERİ

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Firas MOHSEN

REGULATORY COMPLIANCE

"The Effects Of Pth (1-34) And Serm (Raloxifene) Alone, In Sequential Or In Combination Approaches On Osseointegration Of Titanium Implants In Osteoporotic Rabbit model" was prepared in accordance with Erciyes University Graduate Thesis Proposal and Thesis Writing Directive.

PREPARED BY

Firas MOHSEN

SUPERVISOR

Asst. Prof. Dr. Ahmet Emin DEMİRBAŞ

Head of Department

Asst. Prof. Dr. Ahmet Emin DEMİRBAŞ

The study titled “The effects of PTH (1-34) and SERM (raloxifene) alone, in sequential or in combination approaches on osseointegration of titanium implants in osteoporotic rabbit model” was prepared by Firas MOHSEN under the supervision of Asst. Prof. Dr. Ahmet Emin DEMİRBAŞ. It was accepted by our Jury in the department of oral and maxillofacial surgery at Erciyes University Health Sciences Institute as a Ph.D. thesis**.**

……/……/2019

JURI
Signature

Head of Oral and Maxillofacial Surgery Department
Asst. Prof. Dr. Ahmet Emin DEMİRBAŞ

ACKNOWLEDGMENTS

I have been very fortunate to receive plenty of supports throughout the course of my research. I would like to express my gratitude to my supervisors, Professor, who was abundantly helpful and offered invaluable assistance, support and guidance.

Many thanks are due to my friends and colleagues at the,,,,,,, Dental Institute and ……. University. Special thanks to my friend ……. and …… for their help. I would like to express my love and gratitude to my beloved family.

THE EFFECTS OF PTH (1-34) AND SERM (RALOXYPHENE) ALONE, IN SEQUENTIAL OR IN COMBINATION APPROACHES ON OSSEOINTEGRATION OF TITANIUM IMPLANTS IN OSTEOPOROTIC RABBIT MODEL

Firas MOHSEN
Erciyes University, Faculty of Dentistry,
Department of Oral and Maxillofacial Surgery
Ph.D. Thesis, February 2020
SUPERVISOR: Asst. Prof. Dr. Ahmet Emin DEMİRBAŞ

ABSTRACT

Dental implants have come to be a common and frequent treatment choice for tooth replacement. In the clinical success of titanium dental implants, one of the patient-related factors is bone quality. Low bone quality reduces the initial implant stability and histologically affects the bone leading to failure of the desired osseointegration. One of the diseases causing low bone quality is osteoporosis. The aim of the study is to investigate the possibility of reducing the problems experienced in the osseointegration of dental implants with pharmaceutical agents therapy after dental implant surgery and provide a new method for successful implant treatment on the osteoporosis-generated animal model.

60 female New Zealand White rabbits were randomly divided into six groups of 10 animals. The first group (control group) consisted of the animals that did not receive any medication and did not undergo ovariectomy operation. The second group (OVX group) consisted of the animals underwent ovariectomy operation and did not receive any medication. The third group (combined group) consisted of ovariectomized animals receiving combined teriparatide and raloxifene therapy. The fourth group (sequential group) consisted of ovariectomized animals receiving teriparatide and raloxifene therapy sequentially. The fifth group (PTH group) consisted of ovariectomized animals receiving only teriparatide (PTH) and finally the sixth group (Raloxifene group) consisted of ovariectomized animals receiving only raloxifene therapy. Two weeks after the ovariectomy procedure, all groups received intramuscular injections of methylprednisolone acetate (1 mg/kg/day) for four consecutive weeks to induce osteoporosis except the control group. Eight weeks after the ovariectomy process, dental implants were placed in the proximal metaphysis of both tibiae

of all rabbits under general anesthesia. Subsequently, the current drugs were given to the third, fourth, fifth and sixth groups according to the specified methods and the animals were sacrificed 12 weeks after the the dental implant placement. Histomorphometric and the micro CT examinations were performed with the samples obtained from the right tibial bone, and the retraction torque (RTQ) and ISQ (implant stability test) tests were performed with the samples obtained from the left tibial bone. The results were compared and evaluated statistically.

As a result of the RTQ values, the highest value (93.01±27.19 Ncm) was observed in the combined group and the lowest value (49.6±12.5 Ncm) was observed in osteoporosis group ($p=0.015$). In terms of the ISQ values, the mean value of the control group (67.1) was higher than the other groups at the time of the implant placement ($p<0.05$). After the sacrification process, the highest ISQ value was observed in the combined group (76.6). According to the data obtained from micro CT examination, the mean value of bone implant contact of the control group was 40.7% and the OVX group was measured as 24.1%. This difference was shown to be statistically significant ($p <0.05$). The highest bone implant contact value was obtained from the combined group with a value of 41.1% and there was a statistically significant difference between the combined and the OVX group ($p <0.05$). Histomorphometry and micro CT morphometry data were found to support these findings.

In conclusion to our result the combined therapy that involved an anabolic agent with antiresorptive medication is the best way to achieve maximum implant stability and osseointegration in osteoporotic bone.

Keywords: Dental Implant, Osteoporosis, Combined treatment, Sequential treatment, Osseointegration, Teriparatide, Raloxifene

TABLE OF CONTENTS

INTRODUCTION AND PURPOSE

Intra-bone dental implants are frequently used in prosthetic treatment in order to restore the esthetic and functional loss. Dental implants have changed and developed since the past and have become one of the indispensable treatment materials in today's dental practice. It is known that osseointegration plays an important role in the long-term success of implants. Osseointegration refers to the clinical condition in which the intra-bone implant has direct histological contact with the bone and permits functional loading of implant.

The long-term successful use of dental implants by the patient depends on many factors. The level of education and experience of the physician performing dental implant surgery, the amount and the density of the bone in the toothless region where the implant will be placed, the general systemic status of the patient are among the most important factors. The surgeon may not control some factors such as bone quality. Prior to dental implant placement, it is critical to determine bone quality for better clinical results. The quality of the bone plays an important role in the primary stabilization of dental implant which is essential in the success of the implant. Primary stabilization can be defined as implant immobility when placed in the prepared bone in such a way that the implant is not exposed to micro-motions at the time it is first placed. It is known that the micro-movements of the implant will result in the formation of a fibrous membrane at the implant-bone interface resulting in poor osseointegration [1].

One of the diseases that cause the poor bone quality is osteoporosis. Osteoporosis is a disease characterized by low bone density and increased bone fragility [2]. The disease-causing mass loss in the trabecular and cortical bone in general has become an increasingly important problem for public health with the prolongation of human life [3].

The bone is reshaped throughout life by osteoclasts and osteoblasts by the cycle of new bone formation [4]. This cycle makes osteoclasts and osteoblasts distinct targets for pharmaceutical intervention. The goals of osteoporosis treatment are prevention of fracture and skeletal deformities, increasing bone mineral density (BMD) and improvement of life quality. For this purpose, many drugs that increase bone formation (parathormone) and

decrease its resorption (bisphosphonates, denosumab) are used in the treatment of osteoporosis [5]. Drugs used in antiresorptive therapies can only increase BMD to a certain level by reducing the number of osteoclasts and preventing new bone synthesis by osteoblasts [6].

It has been reported that some antiresorptive drugs (bisphosphonates) used alone may cause undesirable side effects in procedures such as tooth extraction, intra-bone implant application. Therefore, combined or sequential medication therapy that are used in the treatment of osteoporosis is on the list and studies on this subject are gaining momentum. The effect of anabolic drugs on bone formation is known but the resorption process does not change with the use of these medications. It is considered that reducing bone resorption with antiresorptive drugs and increasing BMD with concomitant anabolic drugs may be effective in the treatment of osteoporosis. Currently, as a combined or sequential treatment, studies are being performed on the use of PTH and other drugs such as selective estrogen receptor modulators (SERM), bisphosphonates and denosumab. Although there is no significant difference between the single and combined or sequential use for anabolic effect in PTH and SERM drugs, studies have shown that bone resorption decreases significantly in combined use [7].

The aim of this study is to investigate the efficacy of combined or sequential administration of PTH and SERM medication treatment on the osseointegration of dental implant in osteoporotic animal model and compared the results in both healthy and osteoporotic animal models. There are studies showing that the use of certain medication therapy alone has a positive effect on osseointegration after dental implant surgery. The formation of new bone with the combined or sequential medical therapy is important for osseointegration and it is expected to be a more effective treatment modality by stopping bone resorption process which is the main problem. The present study planned to provide new bone formation and decrease the bone resorption process that are required for the success of implant osseointegration. The effects of these treatment modalities on dental implant osseointegration is investigated in this field for the first time.

2. GENERAL INFORMATION

2.1. Dental implants

2.1.1. Dental Implants and types

Dental implants are alloplastic materials placed in the lower or upper jaw with the aim of replacing orofacial structures and tooth loss as a result of trauma, neoplasia and congenital defects[8]. In dentistry dental implants most often contain pure titanium or titanium alloy [8]. Ceramics such as aluminum oxide and other alloys (gold and nickel-chromium-vanadium) are also used as alternative materials[8]. Today, dental implants are frequently used in prosthetic treatment in order to restore the esthetic and functional loss to the patient. Prosthetic restorations with implant support are highly successful treatment choice and have been used as a predictable method in oral rehabilitation[9].

According to the used materials, dental implants are classified as follows[10]:

1) **Metals and Alloys**
- Titanium and Titanium 6-aliminium-4 vanadium
- Cobalt-chromium-Molitaden
- Iron-chromium-nickel

2) **Ceramics**
- Aliminium oxide
- Hydroxylapatite tricalcium phosphate
- Calcium aluminate

3) **Carbons**
- Polycrystal Glass Carbon
- Carbon-silicon

4) **Polymers**
- Polymethylmethacrylate

- Poletrafluoroethylene
- Polyethylene
- Silicone rubber
- Polysulfone

Nowadays, the most pure titanium and alloys (mainly Ti-6al-4v) are used in the construction of implants [11]. In a study by Lauten and Monoghan [12] it was reported that titanium is a biocompatible, bioinert, antibacterial metal. In addition, it was also found that titanium is the most suitable implant material due to its close bone elasticity, low specific gravity and high resistance to corrosion. Today other metals such as gold, stainless steel, chromium-cobalt, which are encountered with problems in biocompatibility and surface properties are no longer used in implant production [8].

Dental implants according to their relationships with the bone are classified as follows;

1. Endosteal implant (Intra-bone)
2. Subperiosteal implant (on bone)
3. Transosteal implant (along bone)[13] (Figure 1).

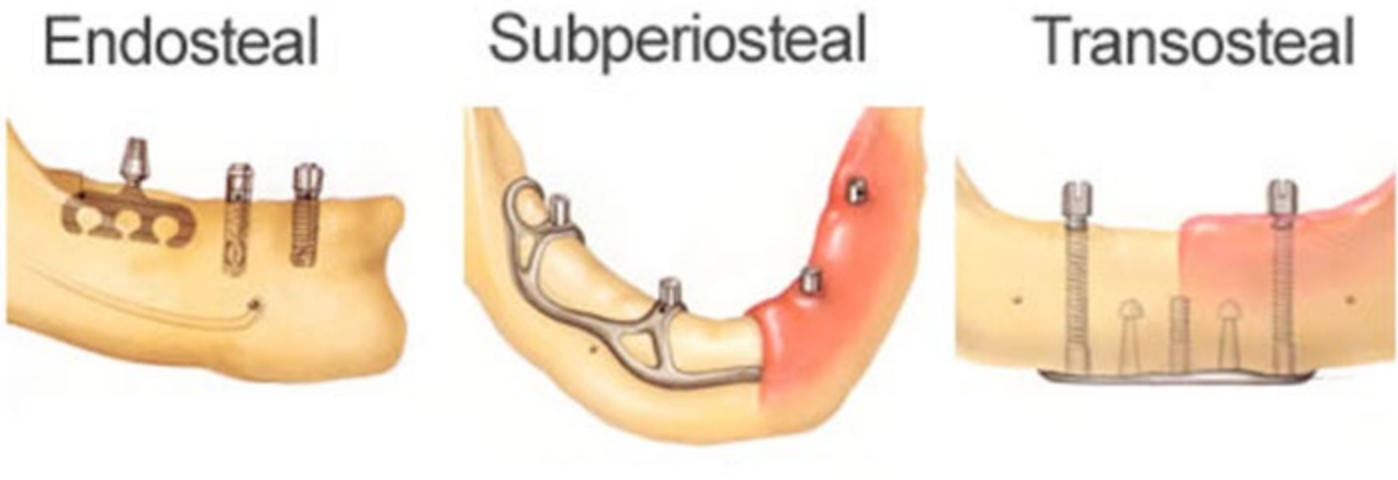


Figure 0-1. Dental implant types according to bone relation

At the same time, a dental implant material must have the following properties[14]:

1. It must be biologically compatible, should not harm the organism.
2. It must be mechanically durable, not corrosive.
3. It should be clinically functional and aesthetic.
4. It should be radioopaque.
5. It should be sterilized.
6. Easily to manipulated.
7. It should not be complicated in surgical and prosthetic terms and easily removable if necessary.
8. It should be economical

2.1.2. Indications and Contraindications of Dental Implants

Proper planning and patient selection are the most important points for the success of dental implant treatment. Therefore, indications and contraindications should be carefully examined and evaluated before implant treatment [15].

Indications of dental implants:

1. Dental implants increase the retention of removable dentures.
2. In patients refused to use removable prosthesis for psychological reasons or are unable to use them due to nausea reflex.
3. Especially in eliminating single tooth deficiencies where neighboring teeth are healthy
4. For the purpose of orthodontic anchorage,
5. It is used as a support for prosthesis after maxillofacial reconstruction.

The cases where the implant applications are contraindicated are divided into two:

General contraindications of dental implants:

1. Systemic diseases that are not under control.
2. Patients with radiotherapy treatment history.
3. People with psychiatric disorders.
4. Bad oral hygiene.
5. Pregnant women.
6. Patients with healing disorders (Ehlers Danlos syndrome, diabetes mellitus, peripheral vascular disease ext).
7. Parafunctions.
8. Smoking and alcohol use (relative contraindication).
9. Age of the patient (patients with growth age).
10. Patients with bone metabolic diseases (Paget, hyperparatroidism, etc.).

Local contraindications of Dental implants:

1. Local bone destruction (osteomyelitis, residual cyst, fibrous bone dysplasia, tumors, etc.).
2. Insufficient bone thickness, height and quality.
3. Leucoplakia in the implant site.
4. Hyperplasia.
5. Maling tumours of the jaw.
6. Insufficient conjoined gum.

For the success of dental implant treatment, the physician should consider and evaluate all these conditions [15].

2.1.3. Dental implants and osseointegration

In 1965 Brånemark introduced implants to dentistry [16]. One of the early definitions of osseointegration was made by Albrektsson et al. and defined osseointegration as a direct

functional and structural connection between living bone and the surface of a load bearing implant [17]. In 1991 Zarb and T. Albrektsson defined osseointegration as a process whereby clinically asymptomatic rigid fixation of alloplastic materials is achieved and maintained in bone during functional loading[18]. After surgical placement of bio-inert material such as titanium into endosteal location, the traumatized bone around these implants begins the process of wound healing. This process can be separated into three typical stages .The first phase is an inflammatory phase, during which local plasma proteins are first adsorbed on the implant surface and a clotting cascade is initiated causing the release of various cytokines from local cellular elements, which regulate adhesion molecule production, increase vascularization rate, enhance collagen synthesis, regulate bone metabolism and activate osteoclasts [19]. This is followed by an acute inflammatory response with neutrophil migration and aggregation 3-4 days after surgery, followed by macrophages becoming the main phagocytic cells present in the wound 5-6 days after surgery. A second proliferative phase is characterized by new vascularization, differentiation, proliferation, activation of cells and formation of an immature connective tissue matrix. During this phase, undifferentiated mesenchymal cells differentiate into fibroblasts, osteoblasts and chondroblasts, of which osteoblasts are responsible for the major part of bone repair[20]. Coupled osteoclast-osteoblast action results in the repair of cortical necrotic border by creeping substitution. Blood vessels enter the necrotic border zone, osteoclasts resorb it and osteoblasts lay new bone around the blood vessels. The healing wound becomes more organized with the passage of time and the fibrocartilaginous callus is transformed into a bone callus. Finally, in the maturation phase, remodeling of the immature bone matrix occurs, and coupled resorption and deposition process of bone continues for many years[21].

Experimental research indicates that both contact osteogenesis and distant osteogenesis may occur around the implant site [22]. The early stage of peri-implant bone healing is very important and involves the body’s initial response to a foreign material and can be categorized into three distinct phases: (1) Osteoconduction is the migration and differentiation of osteogenic cells through a connective tissue scaffold; (2) The second, de novo bone formation, results in a mineralized interfacial matrix and (3) bone remodeling, which also creates bone implant interface comprising de novo bone at discrete sites[22].

2.1.4. Outcomes and factors affecting osteointegration of dental implants

In edentulous and partially dentate patients success rates of dental implants are ranging up to 98% after 10 years[23]. The osseointegration and success rate of dental implant has to meet criteria with respect to function (chewing), tissue physiology (osseointegration), the absence of pain and user satisfaction[24]. Implant survival refers to the dental implant being still in function at the time of examination, regardless of the state of the prosthesis or patient satisfaction and not necessarily meeting all the success criteria[25]. In contrast, implant failure probably results from multifactorial process and is defined when the performance of dental implant measured in a quantitative aspect falls below a specified acceptable limit[26]. Systemic conditions may affect oral tissue by interfering with healing or by increasing their susceptibility to other diseases [27]. There are fairly few absolute contraindications to dental implants treatment [28]. Some relative contraindications and conditions that may negatively affect dental implant results are discussed in the literature such as adolescence, aging, osteoporosis, smoking, diabetes, human immunodeficiency virus infection, cardiovascular disease and hypothyroidism[29-31]. Particularly osteoporosis has been subjected to some debate about the consequences of dental implant therapy [32]. Information from a controlled number of medical studies are complemented by a bigger body of data from in vitro researches and animal experiments. To review these results in a better context, a short evaluation of the bone structure and metabolism will be given, followed by a short resume of the pathophysiology of osteoporosis.

2.1.5. Evaluation of implant success

Albrektsson has identified the factors necessary to ensure a reliable osseointegration as a result of the studies. Those factors are related to the biocompatibility, surface property and the design of the implant, implant placement preparation, the surgical technique used and the loading protocols at the prosthetic stage (40).

The most widely used success criteria today are those described by Albrektsson et al. [33]

and can be listed as follows:

1. Immobility of the implant when tested clinically.
2. Radiolucency around the implant should not be seen radiographically.
3. Marginal bone loss should be less than 1.5 mm in the first year of loading. Vertical bone loss around the implant should be less than 0.2 mm after the first year of implant loading.
4. There should be no sign or symptom of irreversible pain, infection, neuropathy and paresthesia.
5. For an implant to be success, it must meet the above criteria by 85% at the end of the 5-year observation period and 80% at the end of the 10-year period.

2.1.5.1. Radiographic Evaluation

Radiography is the most common method used to evaluate the success of dental implants both before and after surgery. The number and size of implants should be compatible with bone morphology and the anatomical structures. Complex surgical procedures can be planned with the help of preoperative computed tomography (CT) images. This method makes it easier to overcome surgical difficulties and shortens the operation time. In particular, radiographic data and restorative requirements that show proximity to depth and anatomical structures are very important in planning the position, orientation and distribution of implants [34]. Due to the high cost, availability and radiation dose quantity, the use of CT in dentistry is limited. Cone-beam computed tomography (CBCT), developed specifically for the imaging of the maxillofacial region and was introduced in 1996. CBCT, which revolutionized dentistry, has a very low effective dose compared to CT [35]. Therefore, it has become a routine procedure to obtain CBCT images before implant surgery. However, the most important disadvantage of this method is that the image quality decreases due to contrast caused by excessive scattered radiation, especially when the imaging area is large [36].

Conventional imaging techniques have an important role in the evaluation and long-term follow-up of osseointegration. Periapical and panoramic radiographs are the most preferred imaging modalities in clinic practice. Periapical and panoramic radiographs are very useful in assessing the relationship between the implant and the surrounding bone and in the

diagnosis of peri-implant defects. However, because periapical and panoramic radiographs are unable to show the different bone defects such as buccal bone dehisens. CBCT should be preferred in detecting such a condition [37].

Micro CT was introduced by Feld Kamp in the 1980s [38]. Since then, its use has become increasingly widespread. Micro-CT can calculate tissue mineral density, bone mineral density (BMD) and bone volume. In addition, it is accepted as the gold standard for evaluating the three-dimensional structure of trabecular bone. However, it is limited in evaluating the microstructure of cortical bone. Micro CT allows to analysis of the micro-architecture of bone trabeculae and also to measure mineral density.

2.1.5.2. Implant Mobility

Implant mobility is another method in assessing implant stability clinically. Implant mobility can be seen when osseointegration are not achieved and those cases the implant is needed to be removed. Implant stability is defined by both the mechanical stability that are obtained by the compression of the bone that hold the implant and the biological stability that are gained by new bone formation during osseointegration period. The stability immediately after the implant placement is evaluated as primary stability, whereas the stability during function is considered as secondary stability. Implant stability measurements can be performed with devices like : Periotest®, Implatest, insertion torque, extraction torque, percussion test, dynamic model test, Resonance Frequency Analysis (RFA) methods and Osstell® Mentor Device [39].

2.1.5.2.1. Periotest

Originally it was developed to measure the stiffness of the natural dentition and therefore the state of the periodontium; at a later period, it was used in oral implantology to measure the bone/implant interface. It was first used by Schulte and Lukas, as dental measuring device based on the principle of determining the mobility of the implant and the natural tooth by measuring the reaction of peri-implant tissues against a given electrical force [40]. The electrically visualizing striking head strikes the tested object 16 times with an action similar to a retractable ballpoint pen. The entire process takes about 4 seconds. Studies

reported the high degree of repeatability and reliability of this method.

2.1.5.2.2. Implatest

Dario et al. developed this method to monitor the stability of the implant in the digital environment. This method is used mostly in engineering branches [39]. One or more accelerators are adapted to the structure that will be tested [41]. Accelerators are also placed on the recorder that measures acceleration as a function of time. The structure is then struck by a calibrated hammer and the Acceleration Time History (ATH) is recorded by each accelerator. The rate of reduction of ATH indicates the hardness and buffering capacity of the structure [41].

2.1.5.2.3. Reverse torque

Initially it was developed in 1987 by Johansson and Albrektsson. The removal torque test is a highly reliable, objective test method used to assess the quality of the bone-implant connection by measuring the torque required to terminate the connection between the implant surface and the surrounding bone. It has no clinic application and is used normally in animal study experiments [42]. In this method, implant stability is evaluated by measuring the torque value at the breaking point of the bone implant contact.

2.1.5.2.4. Percussion test

The percussion test is a simple technique that can be applied to assess the degree of dental integration. It measures the stability of an integrated dental implant by simply tapping on the healing abutment with the handle of a dental instrument such as dental mirror. Dull sound in percussion is considered to be indicative of soft tissue capsule formation and failure, and high-pitched sound as an indicator of successful osseointegration. The sound changes through the healing process as an effect of increasing implant-bone interface contact. The percussion test provides a user-dependent result which makes this method subjective and unreliable[39, 43].

2.1.5.2.5. Resonance Frequency Analysis (RFA)

This method is based on the theory of vibration. A frequency wave traveling in a steady state and a transducer is used in this technique. This transducer is fixed onto the implant or abutment and the response of the resonance frequency that are sent to the transducer is taken to measure the stiffness of the bone/implant interface by calculating the resonance frequency resulting from the reaction to oscillations applied to the implant bone system [44]. The most used instrument for measuring by this method is the Osstell® Mentor Device (Integration Diagnostics AB; Göthenburg, Sweden). Osstell is a non-invasive instrument used to measure the stability of implants. Implant stability is measured using the SmartPeg placed on top of the implant. The stability of the implant is indicated by the ISQ (Implant Stability Quotient) value read on the instrument screen. The ISQ value ranges from 0 to100. As a result of clinical studies, the acceptable value range of implant stability is found between 55 and 85 ISQ [43, 45].

2.2. Bone

2.2.1. Bone: gross structure, formation

Bone is a composite material made up of 33% organic content and 67% inorganic content and also contain water up to 40%, 35% and 25%, respectively [46]. Its roles consist besides locomotion, include the supporting and the protection of soft tissues as well as acting as a reservoir of minerals. All bones consist of a dense exterior compact bone and a central medullary space which includes trabecular bone [46]. Three unique type of layers is found in adult bones which contain microscopic lamellae. Those layers are identified as circumferential lamellae, concentric lamellae and interstitial lamellae. Concentric lamellae form the majority of compact bone and create its basic metabolic unit [46][Figure 2].

Bone modeling is the procedure which bones form their total size and shape. It begins from embryonic bone development and persists till the preadult period of human growth. Bone are formed on the exterior periosteal surface even though there is bone destruction occurs concurrently within endosteal surface in the course of bone modeling. In the period of

growth and aging, bones increase in length and thickness as bone formation rates surpass bone resorption rates.

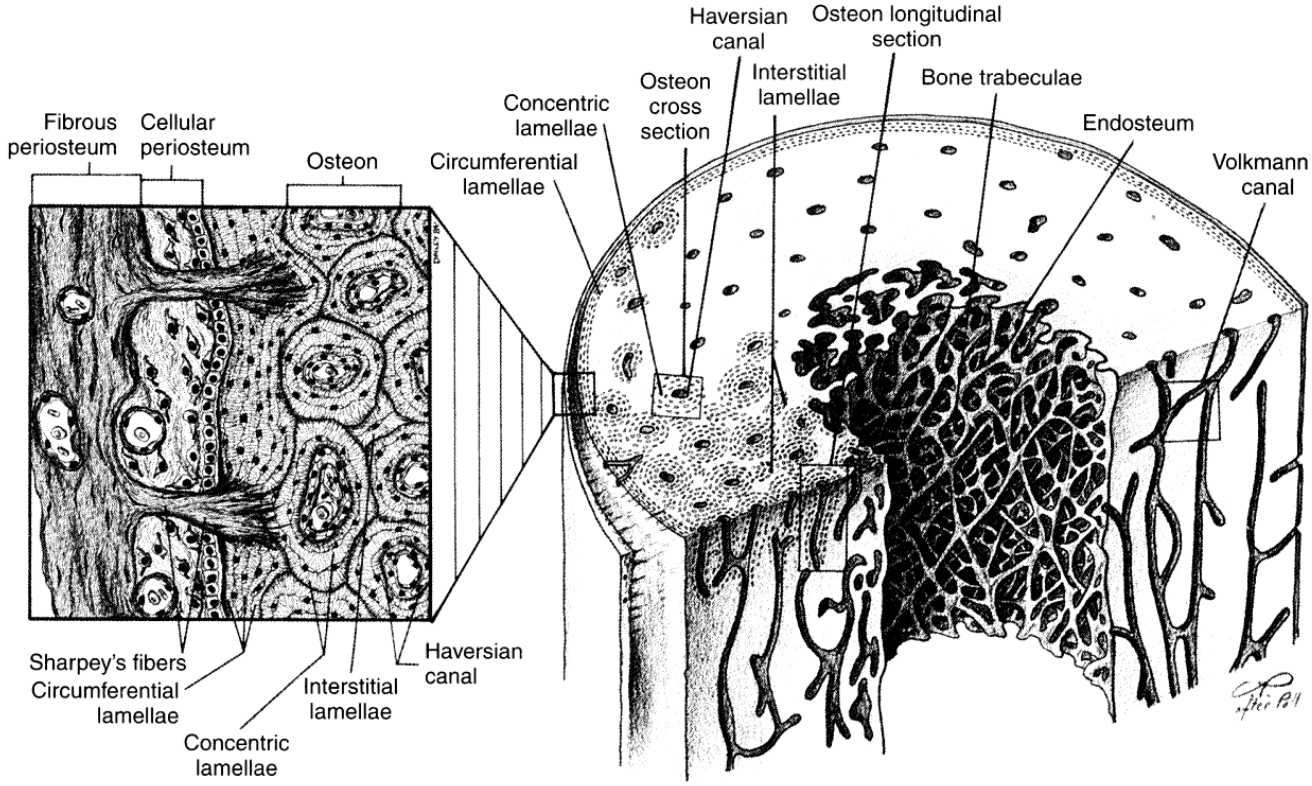


Figure 2. bone structure anatomy

Even though bone is one of the hardest materials of the human body, it is very plastic and in a stable state of remodeling. The increased functional needs lead to new bone formation and the decreased function lead to decrease in volume of bone so is endlessly being resorbed and deposited in reaction to the functional and nutritional demands. Bone remodeling is the replacement of old bone with new bone, in children, bone turnover can be 10 times greater than adults [47]. Through adulthood, bone turnover rates decrease, but in healthy individual, continue to be stable with bone formation being balanced by bone resorption. Most cortical bone, which has an annual turnover ratio of 2% to 10% but trabecular bone of the vertebral column has a greater remodeling rate up to 20% and 30% per year [48].

Bone is formed during life but there is bone loss as a result of aging, body exhaustion and many medical circumstances. The losses of bone is faster with aging, and people that are 80 years of age and in particularly women will lost nearly 30% of their highest bone mass [49]. Histological research has revealed that the volume of intracortical porosities rise in human cortical bones. The outer cortex is less influenced than the inner cortex [50]. Trabecular bone is less resistant to resorption and lost its volume faster than the cortical bone. Over time trabecular bone get perforated because in the end it thin out which lead to separation from its nearby tissue. In the ending of each remodeling cycle small deficits of bone are seen and osteocyte death make remodeling procedure less effective.

Adult have lower bone density and it decreased more with aging and also it become more mineralized which make it less tough and stiffer [51]. Women that are in menopause have a high rate of bone turnover. So it effects the bone by reducing mass end strength which may lead to bone fracture [52].

2.2.2. Bone Remodeling and Regeneration

Bone tissue is in a state of continuous cycling through two processes called modeling and remodeling [53]. Bone structure is the change in bone tissue that occurs during childhood and allows bone growth and skeletal development [53]. Bone rebuilding is a dynamic process that results in the replacement of the old bone with the new bone without significant change in the appearance of the bone after the completion of skeletal maturation. [53].

Bone remodeling is the major metabolic pathway in regulating bone structure and function. The preservation of the bone mass is only possible when the old bone is destroyed, and the new bone is in balance. In a normal restructuring cycle, the amount of bone made is equal to the breakdown. Bone balance; If osteoclast activity is high or the number of resorption areas is increased, osteoblast function is negatively deteriorated which results in loss of bone mass. One of the important control mechanisms in remodeling is the regulation of osteoclast differentiation, activation and survival. Osteoclast differentiation and functions play a critical role in lifelong bone development (Figure 3,4) [54].

Bone tissue within the supporting tissues; represents the greatest achievement in terms of formation and self-renewal capacity [55]. Bone tissue; other than its excellent mechanical properties exhibits outstanding properties in terms of regeneration potential [55]. In case bone fracture, a new bone tissue is formed in the fracture site and it becomes completely normal [56]. In other words, bone, fractures and defects are repaired and regenerated without scarring while maintaining high structural properties (Figure 4)[55].

When a bone fracture happens, bleeding occurs due to tissue damage and a blood clots is formed. The tissue in the fracture area is also not normal and needs to be removed.

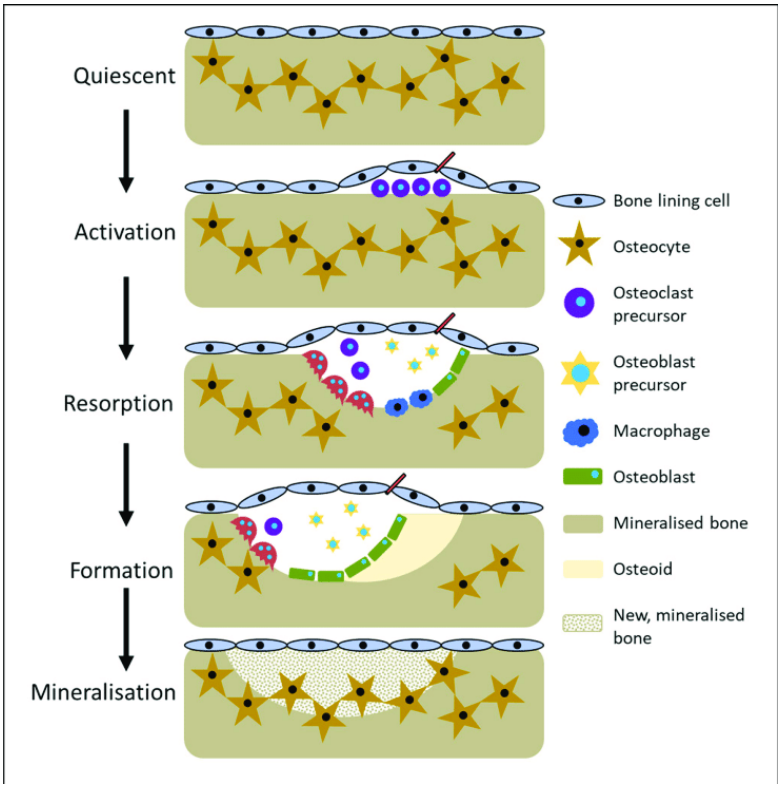


Figure 3. Bone turnover

Neutrophils and macrophages come to the region to eliminate the damaged tissue. Fibroblasts and vessels proliferate over time. This region then becomes a fibrous tissue structure and the fracture site becomes cartilage tissue. This new tissue is called bone callus. In the meantime, the osteoblasts of the periosteum and endosteum in the fracture site multiply and migrate to the fracture site, where they form a cell layer. Then, as in endochondral ossification, primary bone is formed. There is also intra-membranous ossification in the region. Primary bone tissue develops during repair. Tissue is then

gradually removed, leaving the secondary bone to the tissue. Thus, the repaired bone becomes completely normal in that area [56]. The two most important factors in bone healing are mobility absence and a good blood supply [55].

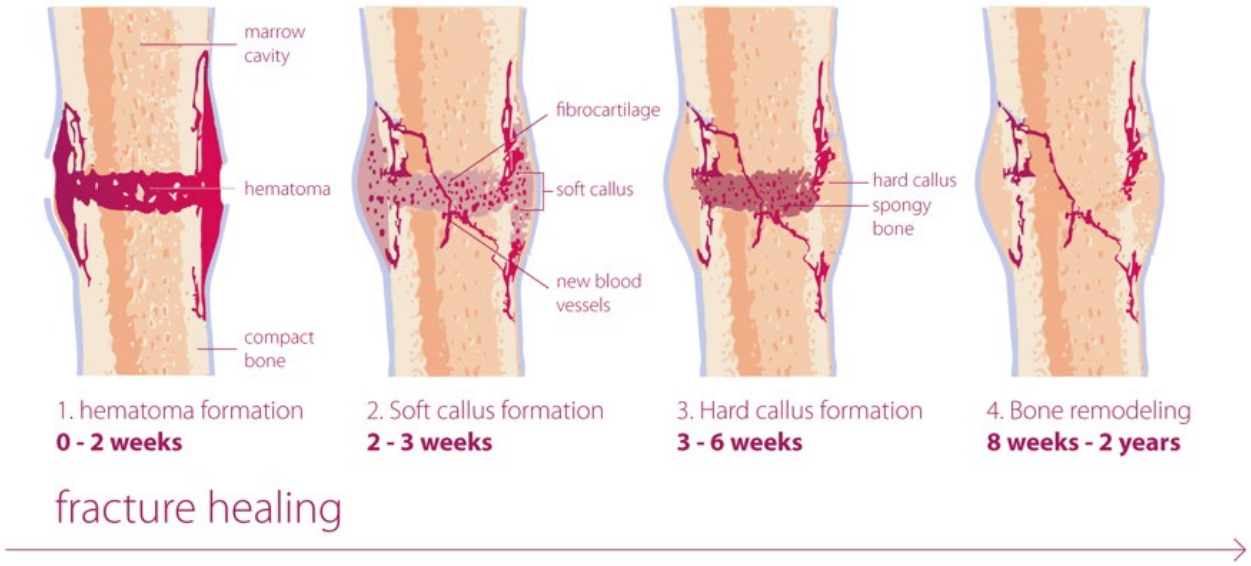


Figure 4. Bone fracture healing

2.2.3. The Classification of alveolar bone

The Alveolar bone is the part of the maxilla and mandible, and it is consisting of two plates of cortical bone divided by a spongy bone. Alveolar bone has a special importance in implant surgery and forms the volume and external structure of the toothless region that implant will be placed [57]. Alveolar bone is classified according to its bone quality and resorption. The negative results of bone resorption after tooth extraction are less noticeable in patients with alveolar bone of appropriate quality and quantity. Resorption is generally more problematic in aesthetic area and in cases of thin alveolar bone prior to teeth extraction[58] .

Several evaluations have been made to classify the alveolar crest caused by bone atrophy. Cawood et al.[59] have classified the alveolar bone after tooth extraction as follows:

Residual ridge form has been classified by Cawood and Howell as follows:

Class I – dentate

Class II – after extraction

Class III – convex ridge form, with adequate height and width of alveolar process.

Class IV – knife edge form with adequate height but inadequate width of alveolar process.

Class V – flat ridge form with loss of alveolar process.

Class VI – loss of basal bone that may be extensive but do not follows predictable resorption pattern.

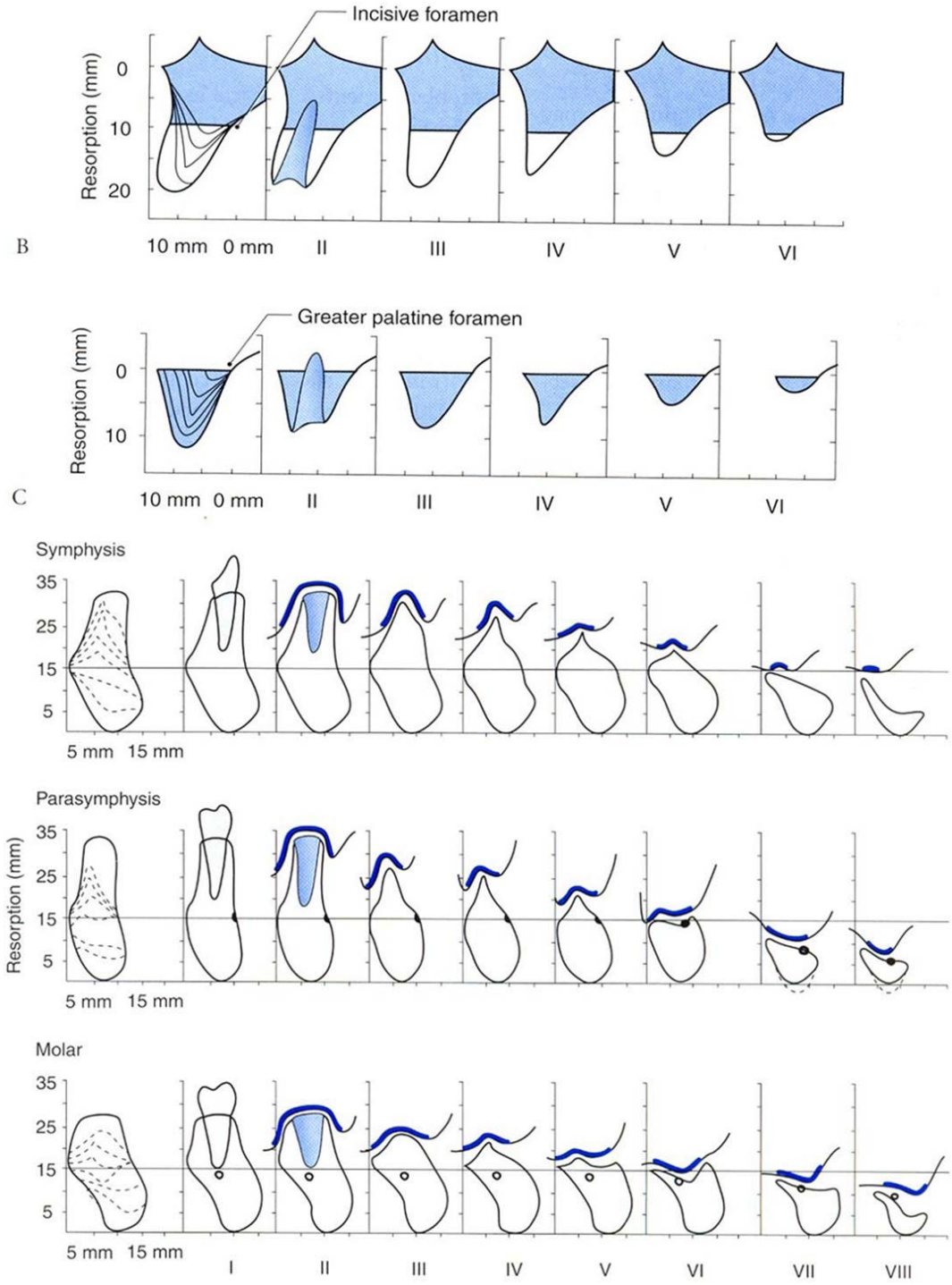


Figure 5. Classification of Residual Ridge Resorption adapted from Cawood and Howell

Lekholm and Zarb classified the jaw bones in terms of bone quality and divided the alveolar bone into 4 classes according to the amount of cortical and cancellous bone (Figure 6) [60]

Type I bone: Consists of a thick compact bone and a small amount of spongious bone.

Type II bone: Consists of thick layer of compact bone around dense trabecular bone.

Type III bone: Consists thin layer of cortical around dense trabecular bone.

Type IV bone: Thin layer of cortical bone around a core of low-density trabecular bone.

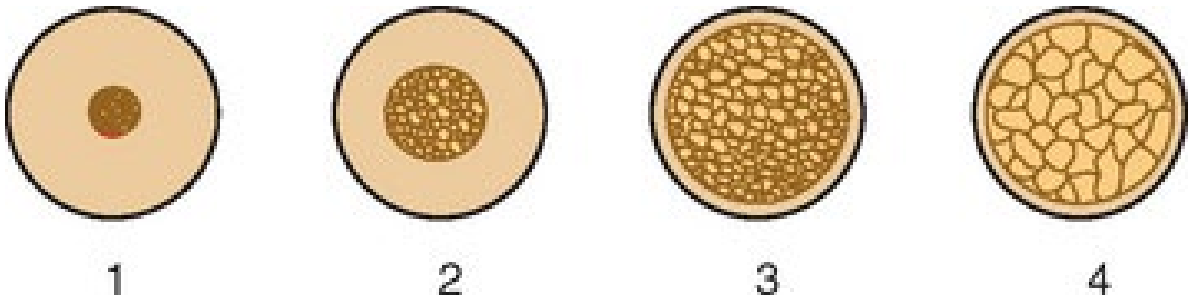


Figure 6. bone classification according to the amount of cortical and cancellous bone

2.3. Osteoporosis

2.3.1. Definition, epidemiology and classification of osteoporosis

Osteoporosis is a multifactorial skeletal disease characterized by an increase in bone fragility due to a decrease in bone volume and also cause negative deterioration in the microstructure of the bone tissue [61].Osteoporosis, derived from the Greek words osteon which means bone and porous which means small holes (pore)[62].This incidence of the disease is increasing especially in postmenopausal women and after ovariectomy procedures due to the decrease in estrogen level. Osteoporosis has become one of the most important health problems with an aging population and longer life span until fractures occur, which causes important secondary health problems. Osteoporosis mainly causes an increase in the fractures of the hip, spine and forearm. Especially it affects the morbidity and mortality in hip fractures.[62].

In epidemiological studies, it is predicted that in the white population that are over the age

of 50 years old, 50% of women and 20% of men will be exposed to osteoporosis in their lives [63]. In another epidemiological study, osteoporosis was found in 6% of men aged 50-84 years and 21% of women in Europe and North America[64]. According to data from the World Health Organization, osteoporosis affects more than 75 million people in Europe, Japan and the United States alone, causing 2.3 million fractures annually, and the lifetime risk of hip, vertebra, forearm fractures is similar to coronary heart disease and It is estimated to be 40% [65].

There have been positive developments in the diagnosis and treatment of osteoporosis. Determining the risk factors for osteoporosis, educating people with risk groups and starting the necessary medical treatments will prevent the occurrence of fractures and reduce the morbidity and mortality associated with osteoporosis. Raising public awareness on this issue will be very useful for the prevention of this expensive, long-term disease.

Osteoporosis has been classified in many aspects such as age, localization, etiology, and involvement of affected bone tissue. (Table 2.1) [66]. According to etiology, primary and secondary osteoporosis is evaluated under two headings.

Table 2.1 Osteoporosis Classification

According to histological appearance	Rapid Cycle Osteoporosis Slow cycle Osteoporosis
According to the bone tissue maintained	Trabecular Osteoporosis Cortical osteoporosis

By localization	General Osteoporosis Local osteoporosis
By age	Juvenile osteoporosis Adult Osteoporosis Senile Osteoporosis
According to the etiology	Primary Osteoporosis Secondary osteoporosis

The cause of primary osteoporosis is not known exactly. In itself, the findings can be evaluated in three groups according to the age of onset. These are: juvenile, idiopathic and involutional osteoporosis [67]. Postmenopausal osteoporosis, which is a type of invasive osteoporosis that are very common and occurs in women after the age of 50 years old. Fracture incidence is more than 40% in osteoporotic patient . The most common osteoporosis-related fractures include vertebral fractures, hip fractures, and wrist fractures. Mortality following hip fracture is mainly attributed to complications such as deep vein thrombosis, pulmonary embolism, pneumonia, deconditioning and poor rehabilitation may cause Mortality rates to increase in up to 20%.[68]. In the etiology of secondary osteoporosis, endocrine, metabolic, hematologic, rheumatic diseases, bone marrow diseases and the use of various drugs may play a role (Table 2.2)[69].

Table 2.2 Secondary osteoporosis Factors

• Endocrine diseases
• Diseases of the gastrointestinal system
• Connective tissue diseases
• Malignant diseases

• Medications
• Diet
• Other reasons

Riggs and Melton introduced the definitions of Type 1 for postmenopausal osteoporosis and Type 2 osteoporosis for senile osteoporosis[70]. Type 1 osteoporosis is seen in post-menopausal women over 50 years old with decreased estrogen, Type 2 osteoporosis is seen equally in women and men in individuals over 70 years old of age. In type 1 osteoporosis, bone loss is greater in trabecular bone than in cortical bone. Bone loss after menopause results from an increase in osteoblastic activity. In type 2 osteoporosis, the trabecular and cortical bones are equally affected [70]

2.3.2. Osteoporosis Diagnosis Methods

For the effective treatment of any disease, it is important to make an Early and accurate diagnosis. Osteoporosis diagnosis is the first step of the treatment. Clinical, biochemical, histological and radiographic techniques are used in identifying osteoporosis. Mostly, the diagnosis is made by dual energy X-ray absorptiometry (DEXA) method based on bone mineral density. Bone mineral density, bone mass and mineral content of the skeletal system are measured. Although DEXA does not show other factors that may affect bone fragility, such as bone architecture and geometry, BMD is a very useful method for predicting bone strength and the fracture risk [71].

Therefore, (BMD) is considered to be the primary measure in determining fracture risk. Many other techniques are used to measure (BMD). These techniques are as follows; Ultrasonic measurement including radiographs, Dual Energy X-ray Absorptiometry (DEXA), Single Photon Absorptiometry (SPA), Dual Photon Absorptiometry (DPA), Quantitative Computerized Tomography (QCT), Speed Of Sound (SOS) and Broadband

ultrasonic Attenuation (BUA) , parameters Single Energy X-ray Absorptiometry (SXA)[72] . The first method used to evaluate the BMD is known as SPA. The SPA method is to measure the regular monoenergetic photon beams made from the light source by radiation from an extremity with a detector containing Na iodide and detecting radiant body glare. This technique can only be used in body areas where soft tissue thickness is constant, which is limited to the distal radius and ulna[73] . Another method is Dual Photon Absorptiometry DPA. Operating principle It is based on the principle of measuring two photon beams with two different energies and the Gadolinium element is used. Whole body, spine, lumbar region and femur can be evaluated with this method. The radiation dose is low. Gadolinium that are used has high disadvantages such as high cost and wrong values. Another preferred method is QCT. The volume of BMD is measured in this technique. The radiation dose is relatively high compared to DPA and is a costly option. It gives an idea about the condition of trabeculae rather than bone density. Ultrasound measurements can also be used to measure BMD. It has advantages such as being low cost, portable and have low radiation dose [74] . The other process used Single X-ray Absorptiometry in the calculation of BMD. In this method, the source is X-ray. The process takes about 5 minutes. It is more cost effective than the other approaches. In this procedure, soft tissue thickness may affect the measurement. For this reason, good results can be obtained if measurements are made in areas where tissue thickness is less. Dual Energy X-ray Absorptiometry is the gold standard today. DXA whole body measurements are based on the principle that dual energy X-rays can determine the mass and volume of any two materials [75].The accuracy of bone mass measurements is very high. For DXA, the average of measurements made in a given healthy population is used as a reference. The x-ray first passes through an adjustment disc containing the absorption material, then through the tissue of the patient and the value obtained from the patient is given by the ratio of the value obtained from the absorption material [74].The whole body measures the anterior-posterior and lateral lumbar spine and femur . It is a two-dimensional imaging method and the radiation dose is low. The parameters taken into consideration in the evaluation of DXA are T and Z scores. The score is derived from the difference between the BMD measurement obtained from the patient and the BMD measured in the young adult population and the standard deviation of the young adult. Z score is obtained by evaluating the standard deviation of the

individual according to his / her age group. In the literature, in the evaluation made with T score, normal values are value of -1 and above, osteopenia between -1 and -2.5 values, osteoporosis if value are -2.5 and below, and severe osteoporosis if fracture is present at -2.5 and below.

World Health Organization (WHO) recognized one principles for the Identification of the osteoporosis and involved having a BMD T-score being more than 2.5 standard deviations below the mean for young healthy adults in the total hip, femoral neck or lumbar spine anatomical regions[76].

2.3.3. Treatment of osteoporosis

There are now several treatment methods for osteoporosis that increase bone density and reduce the incidence of fractures. These drugs can be divided into anti-resorptive agents that inhibit osteoclast activity and anabolic agents that increase bone formation. Anti-resorptive treatments include bisphosphonates, raloxifene. Calcitonin, hormone replacement therapy (HRT), vitamin D and calcium supplements. Teriparatide acts in an anabolic way and strontium ranelate is the first in a new class of drugs, dual acting bone agents (DABAs), that increase bone formation and reduces bone resorption [77].

2.3.3.1. Non pharmacologic treatment

Several non-pharmacologic interventions for the prevention of osteoporotic fractures should be considered for all patients. The attainment of high peak bone mass early in life is one of the most important protective factors against reduced BMD later in life. In addition, strategies to maintain current bone mass for patients in later stages of life should be instituted. Appropriate weight bearing exercise, minimization or elimination of various modifiable risk factors (example; smoking, excessive alcohol intake, maintenance of euthyroid status), and maintenance of adequate calcium and vitamin D intake should be

recommended for all patients[78].

2.3.3.2. Pharmacological Treatment of Osteoporosis

Current pharmacological treatment options in the treatment of osteoporosis include antiresorptive (anticatabolic) strategies that inhibit the resorption of osteoclasts, agents with both antiresorptive and anabolic effects, and anabolic agents such as recombinant forms of parathyroid hormone [79].

Drugs used in the treatment of osteoporosis are[79]:

I. Drugs that Reduce Bone Destruction (Antiresorptive Agents)
- Calcium
- Vitamin D
- Estrogens
- Progestogens
- Selective Estrogen Receptor Modulators (SERM)
- Bisphosphonates
- Calcitonin
- Tibolone
- Ipriflavone
- Denosumab

II. Bone Stimulation Drugs (Anabolic Agents)
- Fluorides
- Anabolic steroids
- Parathyroid hormone and related peptides
- Calcitriol

III. Drugs that stimulate bone formation and reduce bone destruction

• Strontium resin

2.3.3.2.1. Inhibitors of Bone Destruction (Antiresorptive Agents)

2.3.3.2.1.1. Calcium and Vitamin D:

Calcium is the most abundant mineral in the body and is largely stored in bone tissue. It has a fundamental role in the prevention and treatment of osteoporosis. Adults require approximately 1200 mg of calcium per day. Studies have reported that 800 IU / day vitamin D supplementation prevents bone loss, increases BMD slightly and moderately reduces the risk of vertebral and nonvertebral fractures in individuals with vitamin D deficiency[80]. The National Osteoporosis Foundation recommends that every individual aged 50 years and older receive 800-1000 IU vitamin D daily [81].

2.3.3.2.1.2. Estrogen:

Estrogen suppresses osteoclast development, activity, and increases vitamin D receptor numbers in osteoblasts. It also increases Ca absorption from the intestines, increases calcitonin secretion and regulates parathyroid hormone secretion. Thus, estrogen reduces bone turnover, increases BMD and reduces the risk of fractures [82, 83]. The use of hormone replacement therapy (HRT) for 5-10 years; decreases hip, vertebra and arm fractures by approximately 50%, however, when medical treatment is terminated, bone loss reaches the old post-menopausal rate. Due to the potential side effects such as vaginal bleeding, deep vein thrombosis, pulmonary embolism and increased risk of breast cancer in long-term use, HRT is recommended for short-term administration [82, 84].

2.3.3.2.1.3. Selective Estrogen Receptor Modulator (SERM):

SERM depending on the target tissue acts as an estrogen antagonist or agonist. They act on the breast tissue as an estrogen antagonist, as well as on the bone, liver and adipose tissue. Several SERMs are available, such as clomiphene, tamoxifen, raloxifene, bazedoxifene. Raloxifene is the only SERM approved by the American Food and Drug Administration (FDA) for the prevention and treatment of osteoporosis and has been used since 1997.

SERMs provide bone resorption by blocking the production of cytokines that stimulate differentiation of osteoclasts and suppressing osteoclast activation [85, 86]. Side effects are exacerbations of hot flashes, increased risk of venous thromboembolism and leg cramps [87].

The ideal features of SERM should be following these parameters:

• Preserve bone mass and minimize the risk of osteoporosis-related bone fracture by showing estrogen-like effects in bones.

• Reduce the risk of coronary artery asthma by improving lipoprotein properties, eliciting vascular endothelial properties, or similar possible mechanisms.

• Unlike estrogen it should not cause regular or irregular hemorrhage in the uterine endometrial tissue and not increase the risk of endometrial cancer, in contrary reduce it.

• It should not increase the risk of breast cancer and reduce the risk of estrogen positive breast cancer.

• Hot flashes should effectively control menopausal symptoms such as vaginal dryness.

• It should minimize the risk of dementia in elderly women as a result of conventional replacement therapy.

2.3.3.2.1.3.1 Raloxifene

Raloxifene is a drug that acts as SERM that mimics estrogen effects at bones and blood lipid levels without stimulating the breast tissue and uterus (figure 7) (37). Although tamoxifen and raloxifene are compounds of the same rub, the most important difference to distinguishes the two compounds from each other is that raloxifene does not stimulate the endometrium while mimicking the estrogen effects. Tamoxifen acts as an estrogen antagonist on breast tumors and endometrium. The effect of raloxifene on breast tissue is similar to tamoxifen. In other words, it reduces breast tumors [88]. Raloxifene is more sensitive to bone resorption due to estrogen deficiency. One-year use of raloxifene and estrogen has proven to be effective in improving biomechanical properties of the ovarian rat spine and femoral neck. There are two important cytokines and growth factors in signaling pathways in bone resorption. These are IL-6 and TGFβ-3. It has been found that raloxifene shows estrogen-like effects in these signaling pathways [89].

Raloxifene is rapidly absorbed following oral intake. With glucuronidation the liver

undergoes the first transition effect. It participates in enterohepatic circulation. After oral tablet ingestion, bioavailability is about 2% despite absorption of approximately 60%. Achieving mean plasma concentration and bioavailability is dependent on systemic conversion and the incorporation of raloxifene and active glucuronide metabolites into the heterocyclic circulation. Raloxifene is highly dispersed in the body (serum, liver, kidneys, spleen, bone, uterus). Raloxifene and two monoglucuronide conjugates are highly bound (> 95%) to plasma proteins (albumin, α-1 glycoprotein) but not to steroid-bound blood proteins (globulin) [90]. The most common side effects of raloxifene were flushing. Leg cramps, foot and joint swelling, sweating are the most common side effects of raloxifene users. Raloxifene is under the category X of the FDA. Animal studies have shown that there may be miscarriage, cardiac, hydrocephaly, and hormone structure anomaly in the fetus. The use of this drug in pregnant women or those planning to conceive is contraindicated and not recommended [88].

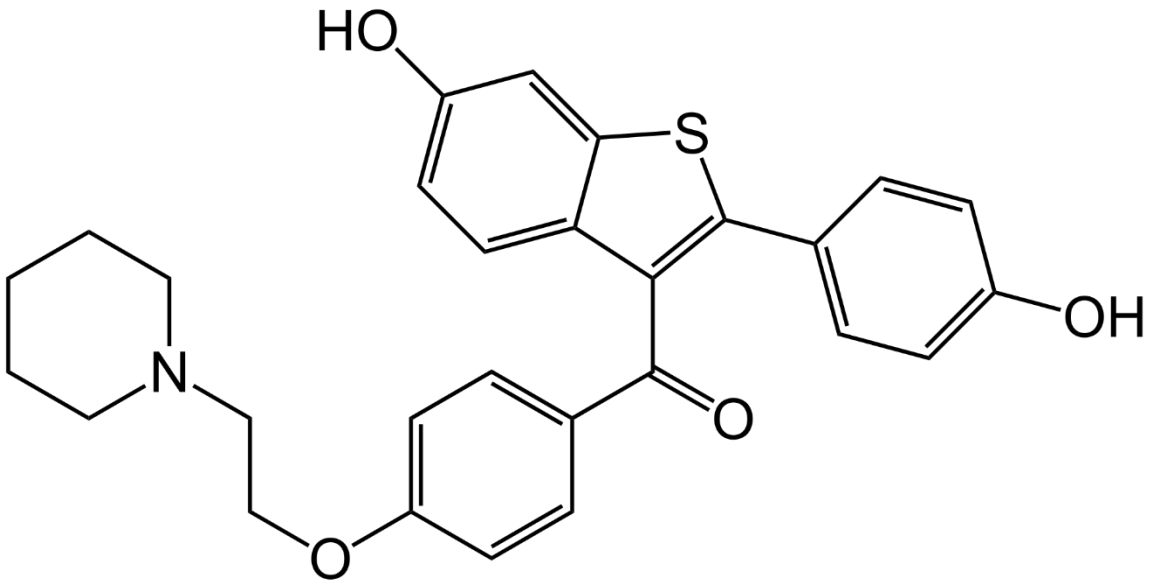


Figure 7. Raloxifene Chemical Structure

2.3.3.2.1.3. Calcitonin:

Calcitonin is synthesized by parafollicular C cells of the thyroid. It binds to calcitonin receptors on osteoclasts and inhibits bone resorption rapidly, temporarily and reversibly. Calcitonin was found to prevent the risk of vertebral fractures but did not prevent non-vertebral bone loss. It has a strong analgesic effect and is used in patients with acute

vertebral fractures. Calcitonin usage has been approved for the treatment of hypercalcemia, Paget's disease, postmenopausal osteoporosis, Sudeck atrophy, and glucocorticoid-induced osteoporosis [91, 92]. However, in 2013 the nasal forms of calcitonin were withdrawn from the market due to the link between nasal calcitonin use and increased cancer risk.

2.3.3.2.1.4. Bisphosphonates (BF):

BFs selectively bind to bone mineral and it is an pyrophosphates analog that are taken by osteoclasts during resorption. BFs cause inhibition and apoptosis to osteoclast activation. Thus, the bone cycle is suppressed and the life of each reconstruction unit is prolonged [93]. It is available in oral, intravenous and in both forms. Either way, it inhibits osteoclast-mediated bone resorption by molecular mechanisms that are activated by intracellular targets. FDA approved alendronate, risedronate, ibandronate, zoledronate in the treatment of postmenopausal osteoporosis. In the FIT 1 (Fracture Intervention Trial) study, it was shown that the incidence of vertebral, hip and wrist fractures decreased by approximately 50% and the risk of multiple vertebral fractures decreased by 90% after alendronate treatment in patients with post-menopausal osteoporosis (PMO) [94] .In the FIT 2 study, it was found that alendronate treatment reduced the fracture rate in the femoral neck by 36% and the rate of vertebral fracture by 44% in patients with PMO without vertebral fractures [95]. In the Vertebral Efficiency with Risedronate Therapy (VERT) study, it was shown that the incidence of vertebral fractures decreased by 41% and the incidence of nonvertebral fractures decreased by 39% after risedronate treatment . Intravenous bisphosphonates are generally well tolerated, the most common side effect being flu-like symptoms such as musculoskeletal pain lasting three days after administration of the drug. In patients receiving long-term bisphosphonate therapy cause side effects such as esophageal damage, atypical fracture of the femur, and rarely osteonecrosis of the jaw may occur. It is contraindicated in patients with hypocalcemia, renal insufficiency, and esophageal stenosis [93].

2.3.3.2.1.5. Denosumab:

Denosumab is a human monoclonal IgG2 antibody that binds RANKL with high affinity and specificity, preventing interaction with RANK on the osteoclast membrane and is a

major mediator of osteoclastic bone resorption, administered by subcutaneous injection every six months. It inhibits the differentiation and function of osteoclast precursor cells. It has been approved for use in postmenopausal women and men who have a high risk of osteoporotic fractures and have not previously responded to other treatments. In the Phase III of (FREEDOM) study, the effects of denosumab on vertebral fractures, non-vertebral fractures and hip fractures were investigated in women with osteoporosis; It was found that it decreased the risk of hip fracture in patients in high risk group and increased the whole body BMD significantly after 36 months of use. Compared to bisphosphonates, it does not bind to bone mineral, its effect is reversible, good compliance with a two injections per year and is not eliminated from the kidney. The risk of hypocalcemia should be considered in renal impairment [96].

2.3.3.2.2. Anabolic Agents That Stimulating Bone Making

2.3.3.2.2.1. Florid:

Sodium fluoride does not reduce the risk of vertebral fracture although it increases BMD. It has been reported that increasing the dose of fluoride decreases non-vertebral fractures but serious gastrointestinal side effects occur at these doses [97].Nowadays it has been withdrawn.

2.3.3.2.2.2. Anabolic Steroids:

Anabolic steroids suppress bone destruction and stimulate bone formation. It was found that bone mineral content and BMD were increased in patients with osteoporosis in the radius and vertebrae with steroid therapy [98] .

2.3.3.2.2.3. Strontium Ranelate:

It is thought that strontium ranelate stimulates bone formation, inhibits bone resorption and is thus effective in bone reconstruction. It reduces non-vertebral and vertebral fractures and also hip fractures [99, 100]. It is indicated in patients with osteoporosis who are unable to tolerate bisphosphonates or where the use of bisphosphonates is contraindicated.

Reported side effects include deep vein thrombosis and skin rash. A significant part of the increase in BMD during strontium treatment is due to the physical effect of strontium on bone tissue. Therefore, the level of increase in BMD does not indicate the same decrease in fracture risk.

2.3.3.2.2.4. Parathyroid Hormone

PTH is produced, stored and released from the parathyroid glands in response to stimulus. The release of parathyroid hormone from the parathyroid gland is dynamic and depends on the extracellular calcium level. An increase in serum PTH level is observed with a decrease in serum calcium levels. PTH has direct and indirect effects on bone metabolism. PTH performs its direct effect by stimulating bone formation by activating osteoblasts and increasing calcium reabsorption and renal excretion from renal tubular cells. PTH shows its indirect effect by enabling 1-α hydroxylase enzyme activation in the kidney and conversion of 25-hydroxyvitamin D, which is the inactive form of vitamin D, to 1,25-dihydroxyvitamin D-metabolite, which is the active form of vitamin D. With the active vitamin D effect, calcium reabsorption from the intestine is increased, thus maintaining serum calcium balance[101] .

2.3.3.2.2.4.1. Anabolic treatment for osteoporosis: teriparatide

There are two forms of human parathyroid hormone PTH 1-84 and PTH 1-34 that are used in the treatment of osteoporosis (figure 8). Teriparatide (TPTD) is the human recombinant preparation of the biologically active N-terminal chain of 34 amino acids of the PTH molecule. The 20-gauge injectable form of this medication was approved in the United States in 2002 for the treatment of postmenopausal in male that had osteoporosis and high risk of fracture and in 2009 for the treatment of glucocorticoid-related osteoporosis. The human recombinant PTH 1-84 form of PTH has been approved in Europe [102]. TPTD and Intact PTH (iPTH) exert their biological effects by revealing specific, G protein-dependent, high affinity cell surface receptors located in osteoblasts and renal tubular cells. Both 2-molecule receptors have similar affinity and therefore produce similar physiological effects on bone tissue and kidneys. Ligand binding to these receptors leads

to activation of the protein kinase 1 cyclic monophosphate, protein kinase C and phospholipase pathways. Activation of these pathways increases the number of activated osteoblasts, decreases in osteoblasts apoptosis, and strengthens bone boundary cells with newly formed osteoblasts to increase bone strength, mass, diameter and thickness [79, 103].The systemic exposure pattern of TPTD determines the effect on the skeletal system. New bone formation on trabecular and cortical (periosteal and / or endosteal) bone surfaces with intermittent administration of TPTD is due to TPTD stimulating osteoblastic activity more than osteoclastic activity[104].The anabolic effects of PTH can be more clearly seen in low-dose and intermittent administration of this medicine. TPTD causes a rapid increase in the levels of bone building markers, followed by a slower and lower increase in bone turnover markers after a period. TPTD stimulates bone formation before bone destruction, resulting in an anabolic effect on the skeletal system, called the anabolic window[102, 105].

Figure 8. Forteo 20 mcg pen (Teriparatide 20 mcg)

2.4. Relationship Between Osteoporosis and Implant Osseointegration

Intra-bone dental implants are commonly used to compensate for the lost chewing functions associated with teeth loss from the lower and upper jaw. Osseointegration was described by Adell et al. [106] as a condition that permits the clinical loading of dental implants and histologically defines direct bone-implant contact [107]. In the studies related

to osseointegration, it was found that the tissue response of the bone to the implant was affected by many factors such as implant surface structure, anatomical region, surgical trauma, and the type of experimental animal. Biologic events at the bone-implant interface should be considered for a good understanding of bone formation around the implant. Many cell types, tissues, growth factors and cytokines play a role in bone formation and remodeling following tissue inflammation in a coordinated manner [108]. In this sense, osseointegration can be described not only as a reaction of bone to implant material, but also as an indicator of the internal regenerative potential of bone [109].

In the literature, the effect of osteoporosis on implant osseointegration and clinical success has been investigated in many experimental and clinical studies. Many researchers and clinicians agree that biomaterial osseointegration is slower in osteoporotic organisms and that the failure of prosthetic implants used in orthopedic and dental reconstructive surgery increases [108, 110, 111]. Examination of the series of biological events during the osseointegration process is important for understanding the effects of osteoporosis.

2.5. Studies on Osteoporotic Animal Model

A study on the animal model is essential to prove the advantage of a new treatment procedure [112]. Preclinical assessment of new medications and procedures is also necessary to confirm their effectiveness and safety[112].

Osteoporosis is a slow-moving disease and due to the difficulties in bone biopsy procedures in human, treatment results cannot be seen in a short time. Because of many factors such as lifestyle, smoking, alcohol use, diet, it is difficult to form homogeneous experimental groups in humans for studies on osteoporosis. Therefore, osteoporotic animal models are frequently used in experimental researches. [112, 113].

Osteoporosis has been induced in various laboratory animals using different methodologies: cortisone administration, denervation, immobilization, absence of gravity, and surgical bilateral ovariectomy that are associated with or without low calcium diets. Bilateral ovariectomy is the method that best reproduces the clinical situation of post-

menopausal osteoporosis [114]. Many animal species have different effects in the process of osteoporosis, but these may be due to differences between the measurement sites or the duration of the experiment [112].

Rat is the most used in animal testing due to their availability. Osteoporotic changes in cortical and cancellous bone usually begin to occur 3 months after ovariectomy in 6-9 months old rats[115]. In addition to these advantages, the rat model has some disadvantages. The main disadvantages are that estrogen deficiency sometimes occurs more than menopause, difficulties in obtaining repetitive blood and bone samples. [115-117]. Besides all this ,difficulties are encountered in fracture and implant treatment procedures because insufficient bone size [118] . On the other hand, the rabbit is used frequently in orthopedic studies due to its convenient bone size, easy supply and homogeneous breeds.[119]. However, their use in osteoporosis studies has been limited.[119]. However, creating experimental osteoporosis model in rabbits can be very useful to investigate the anabolic agents effect on bone, because healing processes, reconstruction rates and bone turnover are quite rapid compared to other species[120]. Osteoporosis can be induced in rabbit by bilateral ovariectomy and corticosteroid injection, resulting in severe trabecular and cortical bone loss in a short time[120]. Skeletal maturity in rabbits is very short after sexual maturity around 5-6 months of age. So postmenopausal effects may occur when ovariectomy is performed from after this time period [119].

3.MATERIALS AND METHODS

3.1. Experimental Animals Selection

A total of 60 New Zealand adult white female rabbits (Oryctolagus cuniculus L) aged 8 months (3.0-3.5 kg body weight) were used in this study. During the study, animals were housed individually and were placed in 50X80X50 cm stainless steel cages. All animals were given unlimited supply off pellets feed and water. Standard adult rabbit pellet feed (Tavaş, Adana, Turkey) was used. At the beginning of the study, all animals were checked by the veterinarian and the animals were randomly divided into 6 groups of 10 (Table 3.1). Power analysis was performed to determine the sample size required in each group and 10 rabbits were seen sufficient for the study. The first group (control group) acted as the positive control group and consisted of animals who did not receive any medication and undergo sham-ovariectomy surgery. All animals in the study underwent bilateral ovariectomy surgery except for the positive control group. The second group (OVX group) acted as the negative control group and consisted of animals that did not receive any medication treatment. The third group (combined group) were administered combined (teriparatide + raloxifene) treatment. The fourth group (sequential group) received teriparatide and raloxifene sequentially. The fifth group (PTH group) consisted of animals receiving only teriparatide and the sixth group (RAL group) consisted of animals receiving only raloxifene medication. Eight weeks after the ovariectomy process, 60 titanium dental implants (Bilimplant, Turkey) were placed under general anesthesia, both in the proximal tibia metaphysis of the animals. The animals in the third, fourth, fifth and sixth groups were then given the available drugs by the determined methods.

Table 3.1 Animal groups distribution

1. 1st Group (N=10 Rabbits) (control group)	Sham ovariectomy
2. 2nd Group (N=10 Rabbits) (OVX group)	Ovariectomy and no medical therapy
3. 3thGroup (N=10 Rabbits) (combined group)	Ovariectomy and the combined drug therapy
4. 4thGroup (N=10 Rabbits) (sequential group)	Ovariectomy and sequential drug therapy
5. 5th Group (N=10 Rabbits) (PTH group)	Ovariectomy and teriparatide therapy
6. 6th Group (N=10 Rabbits) (RAL group)	Ovariectomy and raloxifene therapy

3.2. First Operation (Ovariectomy, Sham-ovariectomy)

Under general anesthesia, fifty animals underwent bilateral ovariectomy surgery, and the remaining animals underwent Sham-ovariectomy procedure and were used as a control group. Animals were fasted the day before the operation. Anesthesia was induced in each rabbit, with intramuscular injection of 50 mg / kg ketamine HCL (Ketalar 50 ml 50 mg / ml), 25 mg / kg xylazine (Rompun 25ml 100mg / ml) and was maintained with an additional dose of 0.2 mL ketamine. This process was repeated if necessary following the same protocol of previous studies[120]. Cefazolin (Cefamezin® IM Flacon, Eczacıbaşı, Turkey) was administered as a preoperative and postoperative prophylaxis and for analgesics 1 mg / kg Diclofenac (Diklofen®, Turkey) were used by intramuscular injection method. After shaving the animal's abdomen, antisepsis was performed using povidone-iodine solution (Poviiodex®, Kimpur, Turkey) (Figure 9). Then, sterile surgical drapes and films (Nepa®, Sterile Drape, Turkey) were adhesively attached to the abdomen of the animals (Figure 10).

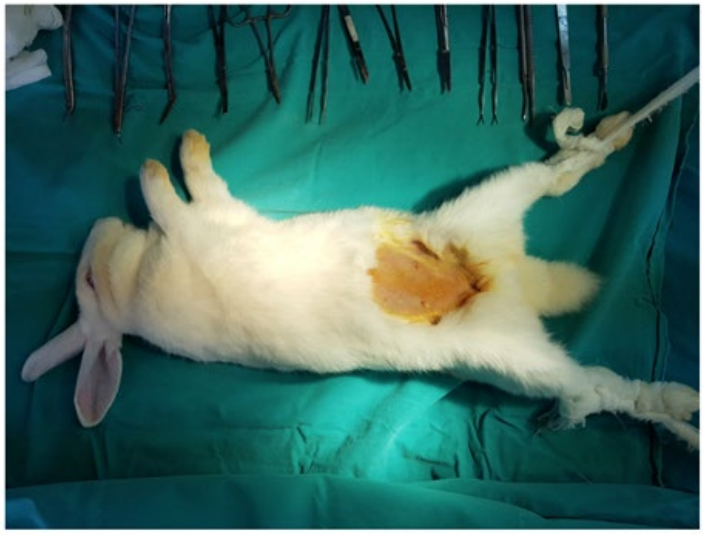


Figure 9. Abdominal shaving and providing antisepsis

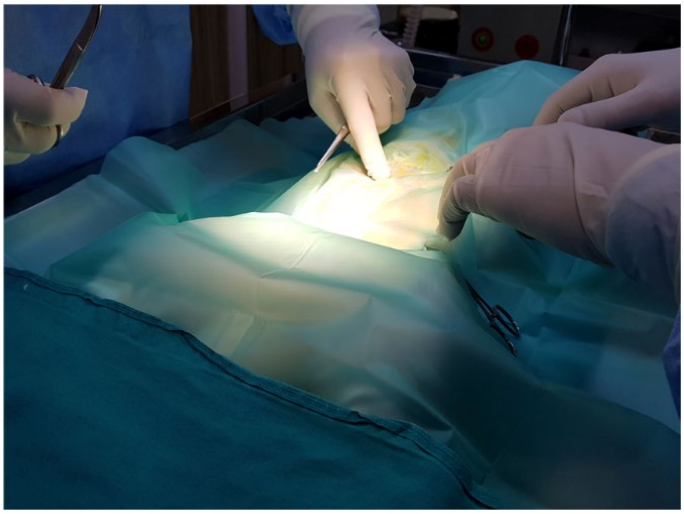


Figure 10. Surgical preparation with sterile surgical drape and film

To performed ovariectomy operation, the abdominal cavity was opened by a 4 cm incision on the midline of the abdomen (Figure 4). After reaching the ovaries, the mesovarium and tuba uterina were ligated by 3.0 resorbable suture (Glikolak®, Ankara, Tukey) and the ovarian tissues were excised bilaterally (Figures 11, 12). For the control group, the abdominal wall was also opened and the ovarian tissues were found but left in place without excision (sham-ovariectomy surgery). In all animals, the abdominal wall, subcutaneous

tissues were sutured using a bioresorbable (suture and the skin was closed using 3/0 non resorbable prolene (polypropylene®, Trabzon, Turkey) suture in layers.
(Figure 5).

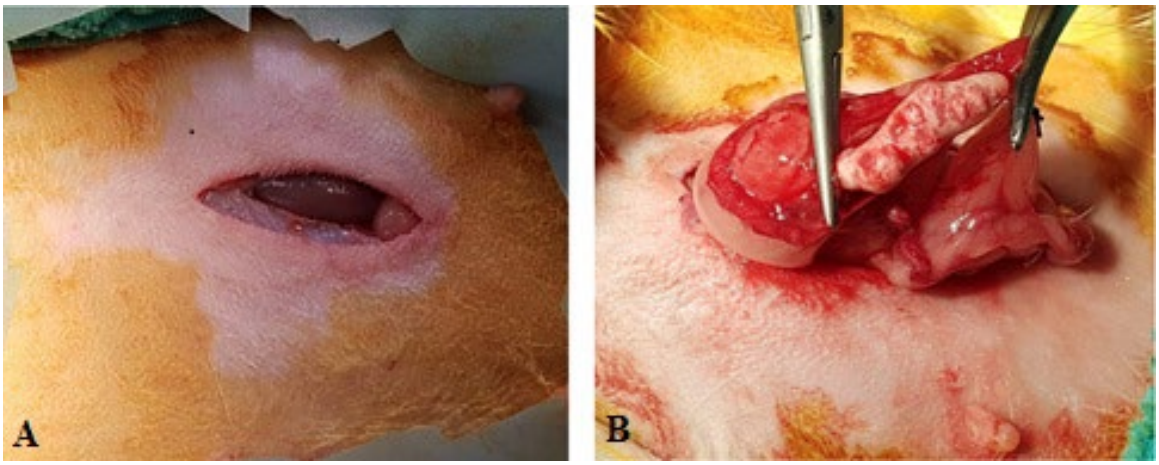


Figure 11. Laparotomy incision and opening of the abdominal cavity (A), Dissection of ovarian tissue after laparotomy incision (B)

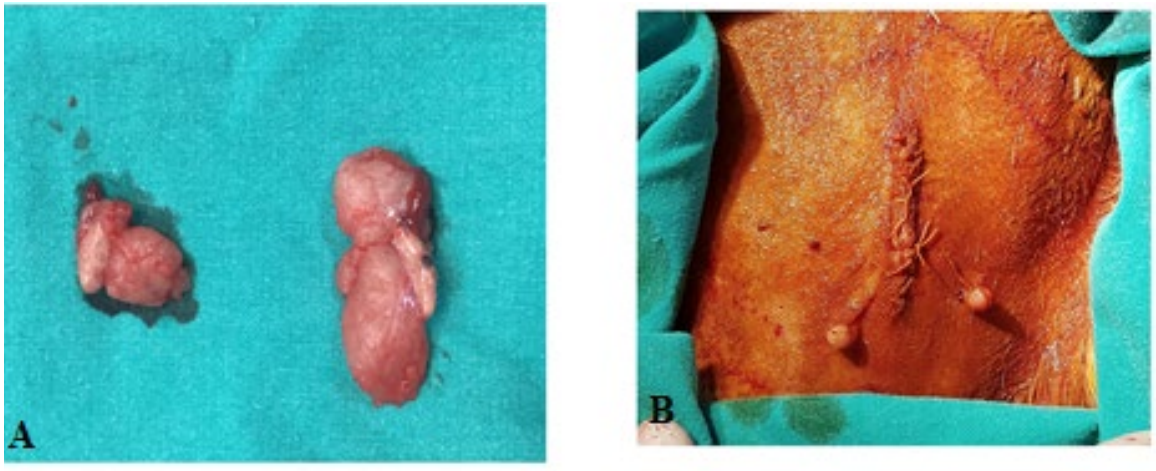


Figure 12. Bilateral excision of ovarian tissues (A), Primary closure of the surgical site (B)

Intra-muscular Cefazolin® 50 mg/kg (Cefamezin® IM Vial, Eczacıbaşı, Turkey) and 1 mg/kg Diclofenac (Diklofen®, Turkey) were injected for three days. Two weeks after the ovariectomy, methylprednisolone (Prednol-1 40 Mg 1 Ampoule) was given intramuscularly 1 mg/kg daily for 4 weeks to accelerate osteoporosis in all animals except the positive control group. It is now known that the combined use of ovariectomy and

steroid administration is an acceptable method to induce osteoporosis in rabbits [120].

3.3. Second Operation (Implant placement)

After 8 weeks of ovariectomy operation, all animals underwent implant surgery under general anesthesia. As implant material a 4,1x6 mm in diameter and length tissue level titanium implant was used (Bilimplant, Turkey) (Figure 13).

Figure 13. 4.1x6 mm SLA surface (Bilimplant, Turkey)

All animals were fasted before the operation and general anesthesia were induced with intramuscular injections of 40 mg / kg Ketamine HCl (Ketalar 50 ml 50 mg / ml) and 5 mg / kg Xylazine (Rompun 25ml 100mg / ml). After shaving the proximal regions of the tibia bone, 2 ml local infiltration of the anesthetic articaine HCL (Ultracaine DS Fort, Aventis Pharma, Turkey) was administered in the operation site per animal. Antiseptic solution was applied to the surgical region following intramuscular prophylactic antibiotic (50 mg/kg cefazolin) and analgesic drug (1 mg/kg diclofenac) injections. Sterile film was adhered to the tibial areas of the animals covered with a sterile surgical drape. Following a 2 cm skin incision extending from the medial to the distal proximal metaphysis of the tibia, the subcutaneous and muscle layers were passed through blunt dissection. The bone surface of the tibial metaphyseal was reached with the help of a scalpel after the periosteal incision (Figure 14). Implants were placed to the prepared wells and the healing heads of the implants were inserted with the aid of a handpiece using a torque force of 10-newton centimeter (Ncm) (Figure 14).

Before placing the healing caps of the implant, primer stability was evaluated with Osstell ISQ device (Osstell AB, Gothenburg, Sweden). Finally, soft tissue was repositioned and

approximated: the fascia was sutured first using a bioresorbable suture (Glikolak®, Ankara, Tukey), the skin was closed using 3/0 non resorbable prolene suture (polypropylene Suture®, Trabzon, Turkey).

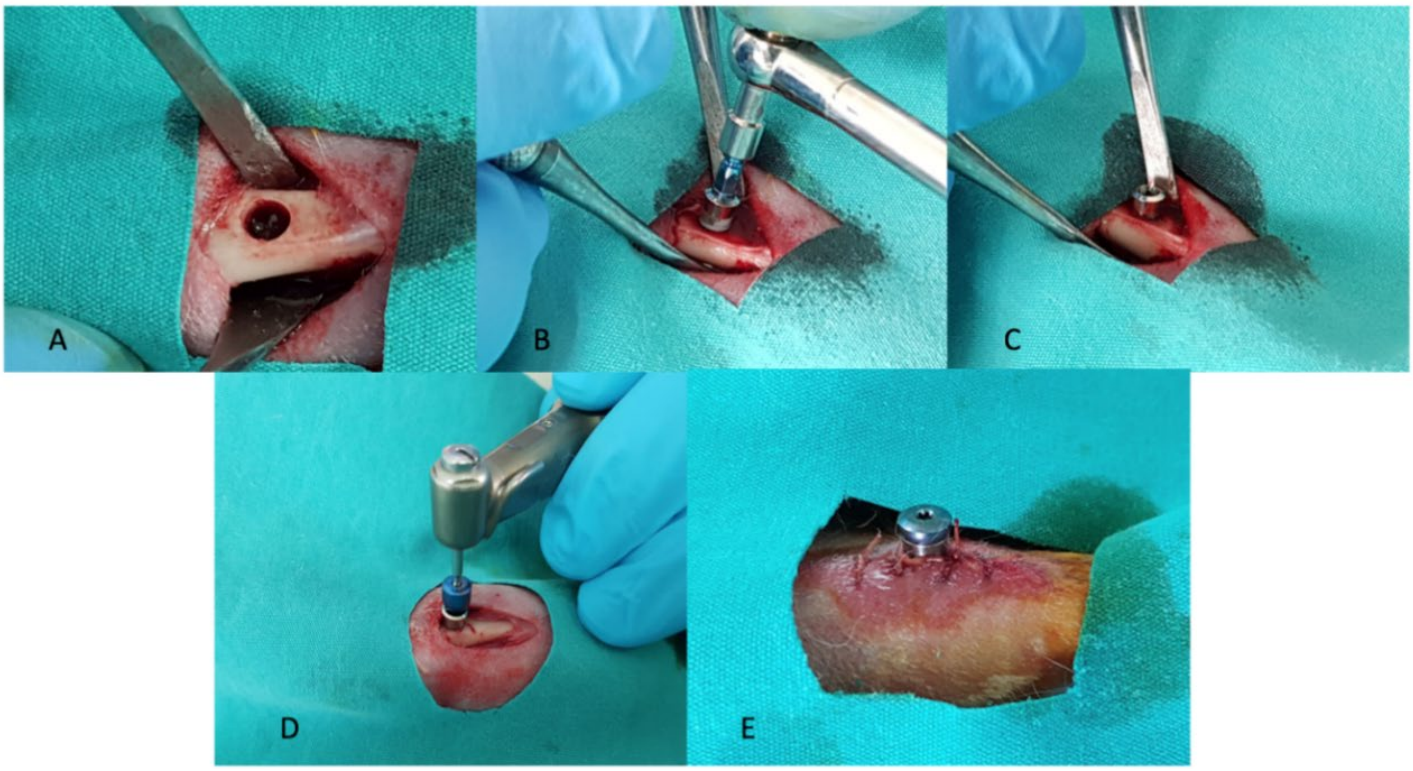


Figure 14. Opening the implant housing (A), Sending the implant with ratchet (B), Completing the implantation process (C), Attaching the implant healing head with contra-angle (D), closure of the surgical site (E)

3.4. Application of Drugs

3.4.1. Third Group (Combined)

In this group, after ovariectomy procedure and implant surgery teriparatide (PTH (1-34)) was administered subcutaneously 10 mg/kg and SERM (Raloxifene) was administered orally 10 mg/kg in combination for 12 weeks.

3.4.2. Fourth Group (Sequential)

In this group, after ovariectomy procedure and implant surgery, PTH (1-34) was administered subcutaneously 10 mg / kg for 6 weeks and after that Raloxifene was administered orally 10 mg / kg for 6 weeks sequentially.

3.4.3. Fifth (PTH) Group

Rabbits in this group were only given PTH (1-34) alone. After ovariectomy and implant surgery, PTH (1-34) was administered subcutaneously 10 mg/kg (5 times a week administration) for 12 weeks.

3.4.4. Sixth (Ral) Group

Rabbits in this group were given Raloxifene alone. After ovariectomy and implant surgery, Raloxifene was administered orally 10 mg/kg for 12 weeks.

Rabbits in the control and OVX groups did not receive any medication treatment and the two groups were identified as positive and negative control groups. For oral administration, the drug was mixed into the drinking water of the animals and was controlled regularly. Animals were sacrificed 12 weeks after the medical therapy for further analyses (Figure 15).

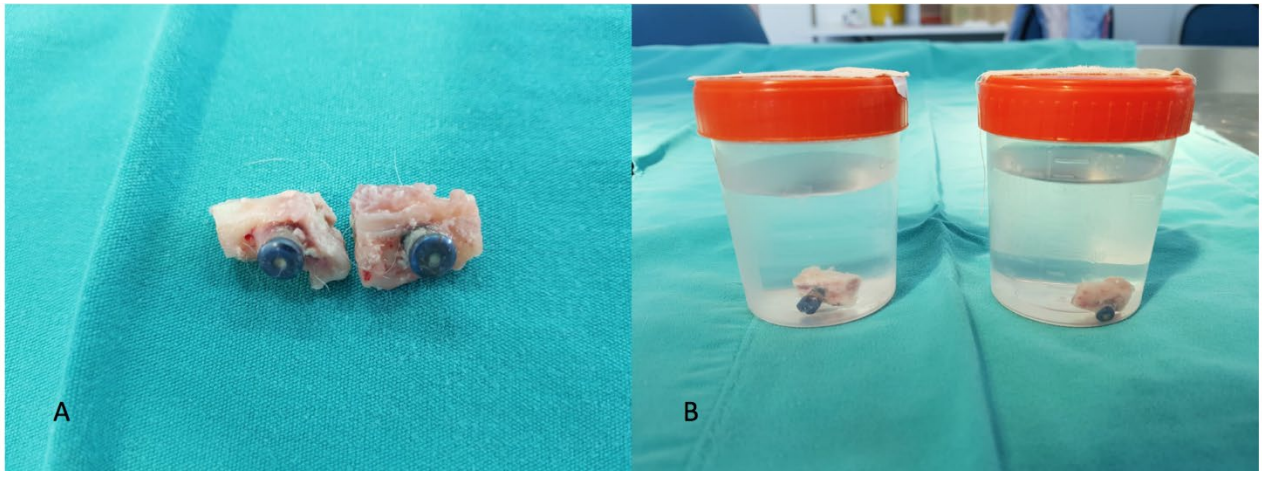


Figure 15. Removal of the bones in which the implants are placed (A), taking samples into containers containing 10% formaldehyde (B)

3.5. Micro-computed tomography (Micro CT)

In this study, Micro-CT (SkyScan-1272, Bruker, Kontich, Belgium) of Hacettepe University Advanced Technologies Application and Research Center (HÜNİTEK) was used in sample scanning (Figure 16). The dental implant and bone contact surface area were examined in 2-3 dimensions and bone density measurements were made about 2 mm around the implant. Before the samples were scanned, the setting of the Micro-CT device was set up to be able to capture and scan all samples, Cu 0.11mm, 0.4-degree rotation state, 13-micron pixel size, 2K resolution and 360-degree shooting, all samples were scanned in those parameters (Figure 9). In the scanning; bone surface density (BS/TV), percentage of newly formed bone volume (BV / TV%), percentage of total porous area (Po (tot) %), and percentage of implant-bone contact surface area (I.S. / T.S. %) of the bone in the examination site were measured. NRecon v.1.6.3 software (Bruker-microBT) was used to convert the data obtained from the samples that was scanned by micro-CT and for analysis CTAn v.1.12 software was used. Images were reconstructed with NRecon 1.6.3 software (BrukermicroBT) and (Bruker® microCT) software using 38 section hardening corrections, 18 ring artifact corrections as well as minimum and maximum contrast limits, resulting in an average of 1800 cross-sections for each sample. 2D and 3D analyzes of samples were performed in CTAn v.1.12 software (Bruker-microCT). The implant and surrounding bone tissue were separated by a multi-level Thresholding procedure (Rüegsegger, Koller et al. 1996, Gabet, Müller et al. 2004). In the analysis phase, the radius setting was set to 20-40 pixels (DPI) for 3D analysis and 20 pixels (DPI) for 2D analysis. An area of approximately 20 pixels (DPI) (2 mm) was analyzed around the implant. CTVol v.2.2.1 software (Bruker-microCT) was used for 3D imaging.

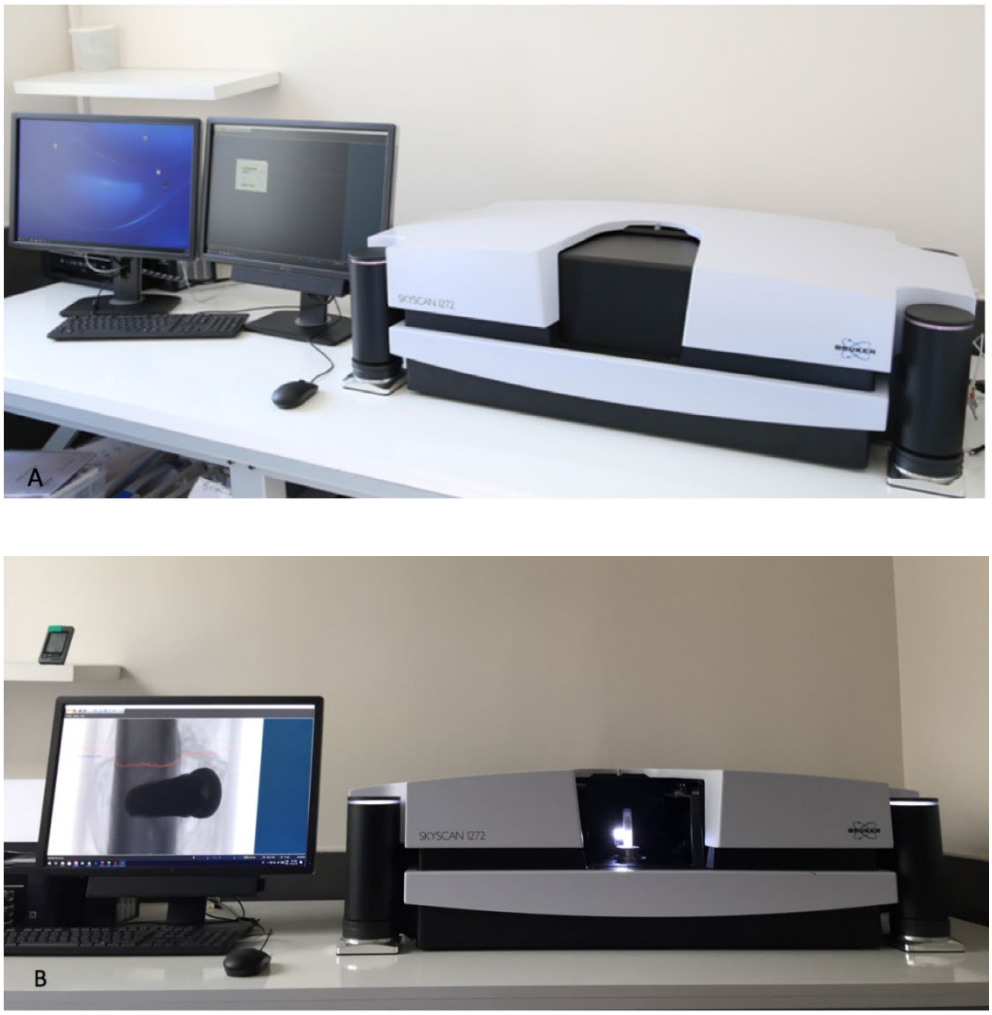


Figure 16. Skyscan 1272 Microcomputer Tomography device (A), Preparation of sample for scanning, insertion into device for scanning, scanning with device (B)

3.6. Histomorphometric Analysis

3.6.1. Preparation of tissues

Implants that was placed on the right tibial metaphysis from each experimental animal were resected with the surrounding bone for evaluation and brought to the laboratory in 4% neutral buffered formalin.

3.6.2. Dehydration process

The samples were dehydrated in 5 pools of alcohol containing 60%, 80%, 96%, 100% ethyl alcohol solution for one day in increasing order. The dehydrated sample were respectively put in 24 hours vacuum and infiltrated with a mixture of 30% methyl methacrylate resin (Tecnovit 7200) and 70% alcohol, then 50% alcohol 50% tecnovit 7200, 70% tecnovit 7200-30% alcohol and finally 100% tecnovit 7200.

3.6.3. Plastic infiltration

The samples were then embedded under vacuum in plastic molds containing methyl methacrylate (Tecnovit 7200) so that no air bubbles remained. These cans that contain the samples were polymerized under light at 40 ° C for 8 hours with a wavelength of 450 nm.

3.6.4. Preparation of blocks for initial cutting and parallel surface preparation

Completely hardened blocks were removed from the transparent boxes to prepare the first cut and prepare the parallel surface. The flat bottom surface was adhered on a plexiglass slide under vacuum using Technovit 7210 VLC (Kulzer & CO. GmbH, Friedricksdorf, Germany).

3.6.5. Sectioning from block

A 300-350 μm in thickness sections were obtained by using a diamond saw (Exakt 300 CL, Exakt Apparatbau, Norderstad, Germany) that are connected to a precision cutting device. These sections were thinned to a thickness of 40 μm with abrasives attached to the micro-abrasion system (Exakt 400 CS, Exakt Apparatbau, Norderstad, Germany).

3.6.6. Dyeing process

Deparaffinized and rehydrated sections were stained with toluidine blue for histological and histomorphometric evaluations and were covered with a coverslip using methyl methacrylate.

The stained sections were examined by Leica DMRB (Germany) light microscope. Sections of all simples in the groups were digitally scanned and recorded with digital

preparation scanning software (Microvisioneer, Germany). The obtained digital sections were analyzed by Sedene-Pathcore (Canada). Measurements were made in actual length units of a millimeter [mm] and micrometer [µm]. The percentage of bone-implant contact (BIC) measurements were performed on the same site of the bone that implant was placed using the Sedeen Digital Cross-Section program (Figure 17). For this, the following formula was applied [121].

$$BIC = \frac{\text{Bone-Implant Contact Length}}{\text{Peripheral Length of the entire implant}} \times 100$$

The result of each group was compared, and statistical analysis was performed.

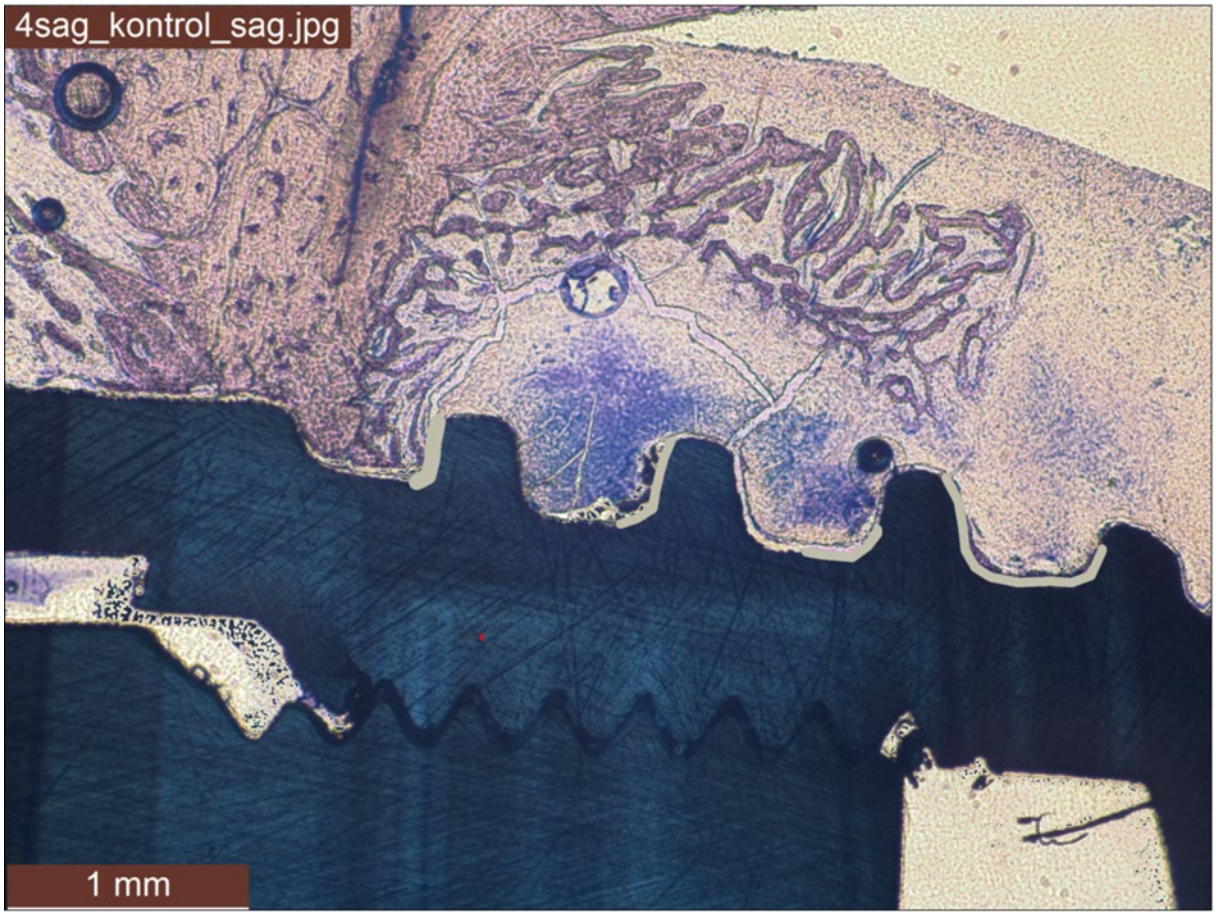


Figure 17. Screenshot from histomorphometric analysis software. Measuring the surface area of the dental implant within the bone.

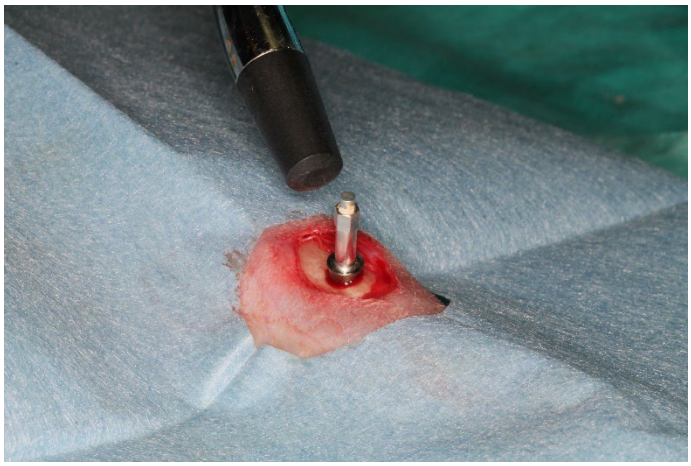
3.7. Biomechanical Tests

3.7.1. ISQ (Implant Stability Test)

Resonance frequency analysis (RFA) was performed using an Osstell® device (Figure 18,19). Osstell device is a wireless measuring device developed for use in dental applications. Osstell (Classic) involves the use of a small transducer that acts as an electronic tuning fork attached to an implant or support. The device operating principle is based on the vibration of the specially designed SmartPeg or transducer in a low range (less than 1mm displacement). The transducer is connected to the frequency response analyzer on the other side and makes the measurements. Response measurements are indicated as resonance frequency (Hertz) and ratio.

A transducer is a small electronic circuit that is the most essential part of the system. The screw is attached to the body of the implant and the other end is connected to the device. It is made from stainless steel or titanium and includes a small bridge. The osstell device has a screen that can display graphics[122]. It has a 15-hour operating time and is powered by a rechargeable power supply. The results are graphically reflected on the display of the device as well as numerical ISQ values. The memory of the device may store up to 32 different measurements without transferring data to the computer. The transducer is stimulated via the factory-programmed frequency response analyzer and shows micromotion.

Figure 18. Osstell Device



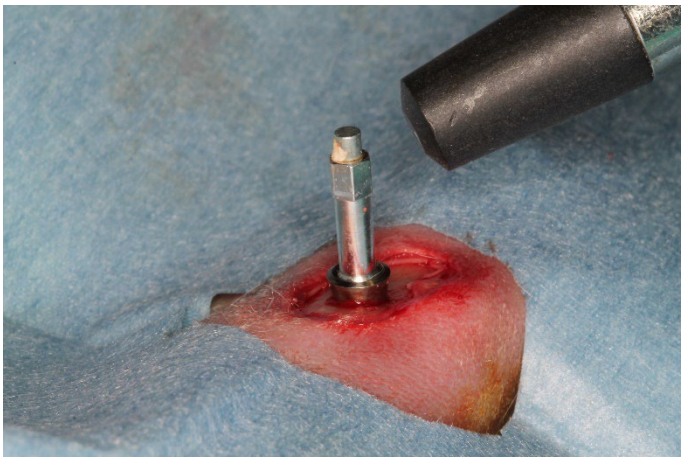


Figure 19. Measurements of ISQ were performed twice, immediately after implant placement and after scarification procedure, and the results were statistically compared.

3.7.2. Removal Torque Test

Evaluations of the removal torque were made after the sacrification of all animals. The tibial bones and implants were resected together and removed. The extracted samples were wrapped and soaked in physiological saline and stored at -20 C until test time. The digital torque meter of Erciyes University Faculty of Dentistry Research Laboratory was used to evaluate the removal torque (MARK-10 MTT01-12, New York, USA) (Figure 20, 21). All values of the device have been reset after each operation. The healing abutments of all implants were removed then the transfer parts and ratchets that was sent from the implant company were used. When an implant was unscrewed, a rupture between bone and implant occurred so the peak torque value fell quickly. Up to this moment, no macroscopic movement of the implant was evident. After rupture, implant unscrewing required low torque. On the digital display of the device, the torque was automatically recorded in Newton/centimeter units (Ncm). The procedure was repeated for all samples.

Figure 20. MARK-10 Model MTT01-12 digital torque meter

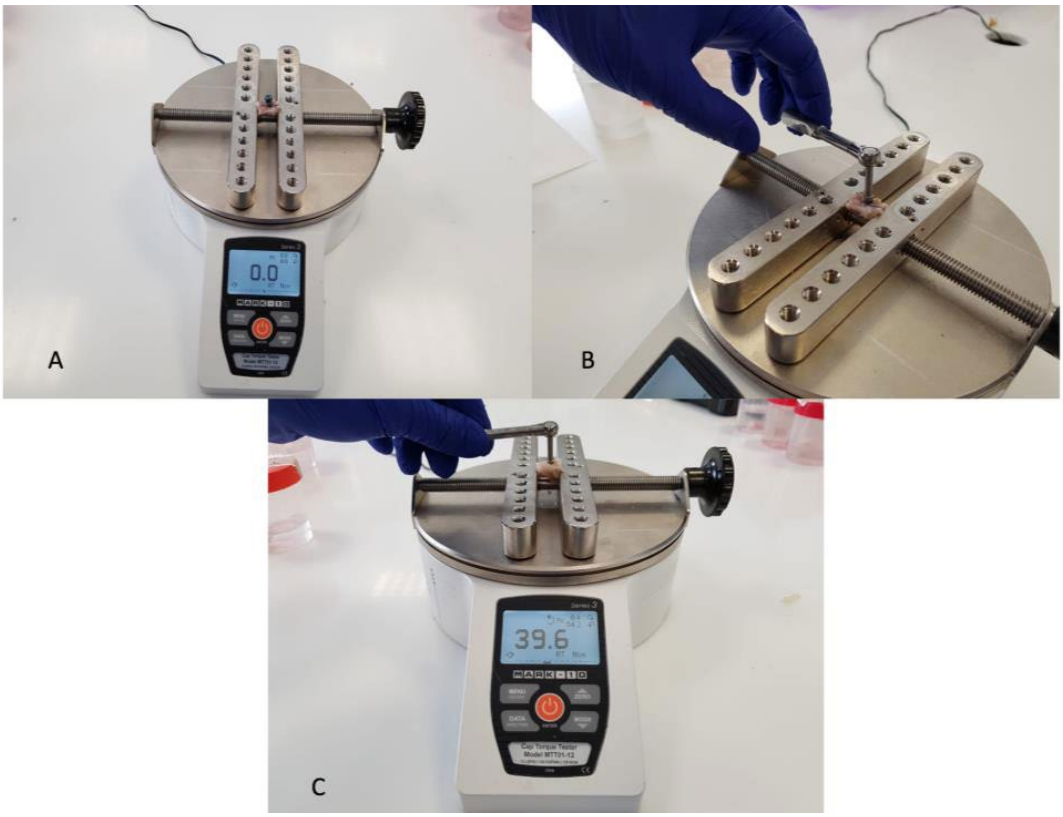


Figure 21. Placement of samples into the device (A), application of the removal torque with a ratchet (B), recording of the obtained value (C)

3.8. Statistical Analysis

For statistical analysis, Turcosa Analytical Software (Kayseri, Turkey) was used. Shapiro-Wilk test was performed to test the normal distribution of the data. One-way ANOVA test was used for multiple comparisons of the normally distributed data, and the Kruskal Wallis test was used for non-normally distributed data. As a result, P values of less than 0.05 were regarded as statistically significant.

4.Findings

During the study, two rabbits in the combined group were lost due to unrelated causes. In the sequential and PTH groups, one rabbit was lost due to infection because of tibial fracture after implant surgery and was sacrificed and excluded from the study. In the RAL group, one rabbit died as a result of an infection after ovariectomy and in the OVX group, two rabbits died as a result of an infection due to tibial fracture after implant surgery.
As a result, seven rabbits were sacrificed and excluded from the study. As total 53 rabbits were used in this study.

4.1. Removal Torque Findings

Since the data were distributed normally in the removal torque findings, the One Way Anova test was performed to analyze the value difference between the groups. The mean removal torque values of the control and OVX groups were 76.2 ± 19.6 Ncm, 49.6 ± 12.5 Ncm, respectively. The mean removal torque value from the combined group was found to be the highest (93.01 ± 27.1 Ncm) and the difference between the combined group and the control group did not show statistically significant differences ($P = 0.7$). The difference between the combined group and the OVX group was statistically significant ($p = 0.015$). The mean values of the remaining groups were close to each other but did not show statistically significant differences ($p > 0.05$).
The highest, lowest and average removal torque values parameter are shown in Table 4.1 and the statistical analysis of the difference between the groups is shown in Table 4.2.

Table 4.1. Removal torque highest, lowest and average values according to groups.

Groups	Highest Torque Value (Ncm)	Lowest Torque Value (Ncm)	Average Torque Value (Ncm) ± Standard Deviation (SS) (N)
Group 1 (Control)	99.3 Ncm	36.6 Ncm	76.2±19.6 Ncm (10)
Group 2 (OVX)	65.6 Ncm	31.2 Ncm	49.6±12.5 Ncm (8)
Group 3 (Combined)	147.1 Ncm	54 Ncm	93.01±27.19 Ncm (8)
Group 4 (Sequential)	125.9 Ncm	33.5 Ncm	78.4±35.8 Ncm (9)
Group 5 (PTH)	137.6 Ncm	34.1 Ncm	74.8±29.5 Ncm (9)
Group 6 (RAL)	104.3 Ncm	48.4 Ncm	78.5±19.1 Ncm (9)

Table 4.1. Statistical difference between the removal torque values of the groups

* Statistically difference ($p < 0.05$).

Difference of removal Torque Averages	Value difference (Ncm)	p value
Group 1 (Control)-Group 2 (OVX)	24.6 Ncm	0.24
Group 3 (Combined)-Group 1 (Control)	16.7 Ncm	0.72
Group 4 (Sequential)-Group 1 (Control)	2.1 Ncm	1.00
Group 1 (Control)-Group 5 (PTH)	1.43 Ncm	1.00
Group 6 (Ral)-Group 1 (Control)	2.28 Ncm	1.00
Group 3 (Combined)-Group 2 (OVX)	43.38 Ncm	***0.015*** **
Group 4 (Sequential)- Group 2 (OVX)	28.8 Ncm	0.19
Group 5 (PTH)-Group 2 (OVX)	25.2 Ncm	0.32

Group 6 (Ral)-Group 2 (OVX)	28.9 Ncm	0.19
Group 3 (Combined)-Group 4 (Sequential)	14.5 Ncm	0.8
Group 3 (Combined)-Group 5 (PTH)	18.1 Ncm	0.6
Group 3 (Combined)-Group 6 (Ral)	14.45 Ncm	0.8
Group 4 (Sequential)-Group 5 (PTH)	3.6 Ncm	1.00
Group 6 (Ral)-Group 4 (Sequential)	0.11 Ncm	1.00
Group 6 (Ral)-Group 5 (PTH)	3.7 Ncm	1.00

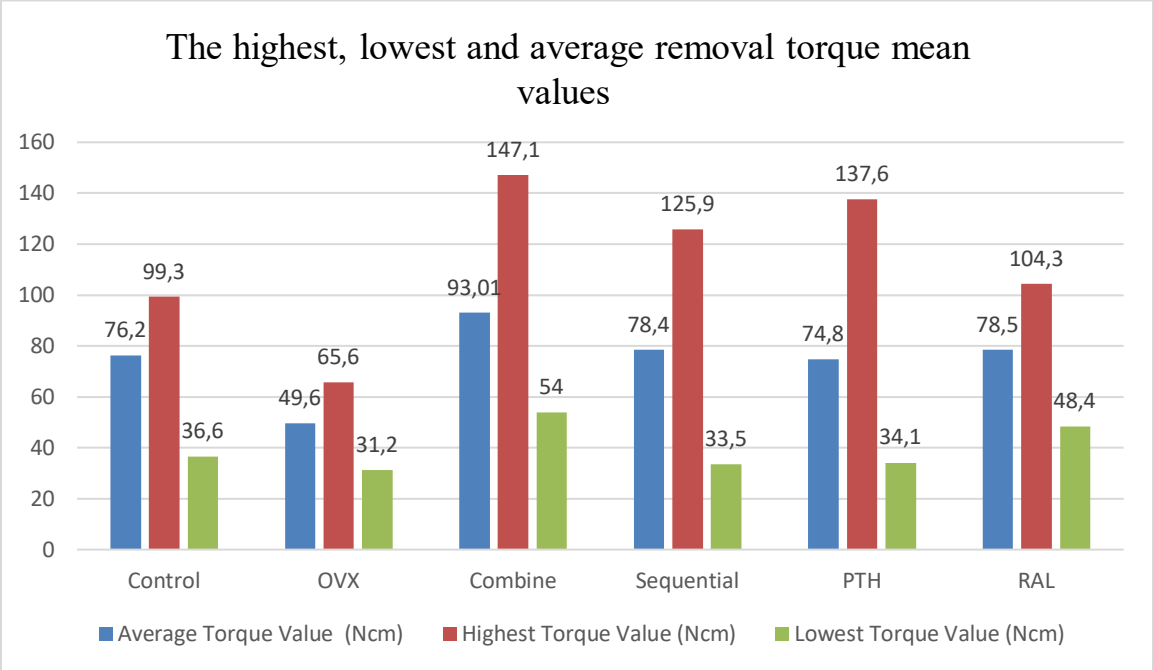


Figure 22. The highest, lowest and average removal torque mean values of the experimental groups.

4.2. ISQ (Implant Stability Test)

Resonance frequency analysis (RFA) was performed using an Osstell device. ISQ values were recorded on the day of implant surgery (T0) and after the scarification procedure (T1). In the statistical analyses, the T-test was used for intra-group examination. The highest T0 values were obtained in the control group (67.1 ± 3.4) and value differences were shown to be statistically significant from the other groups ($p < 0.05$) excluding the third and fourth group averages value. The lowest value was obtained in the second group (OVX group) (61.4 ± 3.8) and apart from the control group, value differences between groups were not statistically significant ($p > 0.05$). The highest T1 mean value was obtained from the combined group (76.6 ± 3.8), and only value differences between the combined group and the OVX group were statistically significant ($p < 0.001$).But the difference between the other groups was not shown to be statistically significant.

Value differences between the mean T1 and T0 from ISQ values of all implants were analyzed statistically. There was a statistically significant increase in T1 values in all groups compared to T0 values ($p < 0.05$). The highest increase was seen in the RAL group ($p < 0.001$). This increase was followed by the combined group and the PTH group, respectively ($p < 0.001$). The groups with the lowest increases were found in the control and OVX group. The highest mean value increases were observed in the groups receiving medication treatment. ISQ values and statistical analysis results are given in Table 4.3 and Table 4.4.

Table 4.3. The difference between the mean values between T0 and T1 values of all groups was statistically significant.

	T0 (Average)	**T1 (Average)**	**T1-T0 (Difference)**	***P***
Control group	67.1	73.9	6.7	<0,001*
OVX Group	61.4	68.9	7.5	<0,001*
Combined Group	62.9	76.6	13.7	<0,001*

Sequential Group	63.5	75.9	12.4	<0,001*
PTH Group	61.9	74.8	12.9	<0,001*
RAL Group	61.8	76	14.1	<0,001*

Table 4.4. Comparison ISQ mean values between groups T1 and T0

T1 (Sacrification Day) Values	**Difference Between Means**	***P***
Group 1 (Control)-Group 2 (OVX)	4.96	***0.013****
Group 3 (Combined)-Group 1 (Control)	2.78	0.6
Group 4 (Sequential)-Group 1 (Control)	2.04	0.9
Group 1 (Control)-Group 5 (PTH)	0.9	1
Group 6 (Ral)-Group 1 (Control)	2.1	0.8
Group 3 (Combined)-Group 2 (OVX)	7.75	***<0,001****
Group 4 (Sequential)- Group 2 (OVX)	7.006	***<0,001****
Group 5 (PTH)-Group 2 (OVX)	5.95	***<0,001****
Group 6 (Ral)-Group 2 (OVX)	7.06	***<0,001****
Group 3 (Combined)-Group 4 (Sequential)	0.7	1
Group 3 (Combined)-Group 5 (PTH)	1.7	0.9
Group 3 (Combined)-Group 6 (Raloxifene)	0.68	1
Group 4 (Sequential)-Group 5 (PTH)	1.05	1
Group 6 (Ral)-Group 4 (Sequential)	0.05	1
Group 6 (Ral)-Group 5 (PTH)	1.11	0.9

T0 (Operation Day) Values	Difference Between Averages	*P*
Group 1 (Control)-Group 2 (OVX)	5.73	***0,001****
Group 3 (Combined)-Group 1 (control)	4.2	0.06
Group 4 (Sequential)-Group 1 (control)	3.6	0.16
Group 1 (Control)-Group 5 (PTH)	5.2	***0.004****
Group 6 (Ral)-Group 1 (control)	5.2	***0.003****
Group 3 (Combined)-Group 2 (OVX)	1.4	0.9
Group 4 (Sequential)- Group 2 (OVX)	2.06	0.9
Group 5 (PTH)-Group 2 (OVX)	0.5	1
Group 6 (Ral)-Group 2 (OVX)	0.4	1
Group 3 (Combined)-Group 4 (Sequential)	0.5	1
Group 3 (Combined)-Group 5 (PTH)	0.9	1
Group 3 (Combined)-Group 6 (Ral)	1.01	1
Group 4 (Sequential)-Group 5 (PTH)	1.5	0.9
Group 6 (Ral)-Group 4 (Sequential)	1.6	0.9
Gorup 6 (Ral)-Group 5 (PTH)	0.5	1

4.3. Micro-CT Findings

The high-resolution 3D images obtained from micro-CT clearly demonstrated differences amongst the six groups. Analysis of variance one-way ANOVA test was used for statistical evaluation because data followed a normal distribution. According to the result, the mean percentage bone-to-implant contact (BIC%) in the control and OVX group was found to be 40.7% and 24.1%, respectively. In medication groups, the mean percentage bone-to-implant contact (BIC%) values of the combined, sequential, PTH and raloxifene group was 41.1%,28.5%, 32.2% and 32.05%, respectively (Figure 16). The lowest value was obtained from the OVX group, while the highest value was obtained from the combined group. The difference was shown to be statistically significant ($p < 0.001$). The mean value percentage of the groups receiving medication were close to that those of the control group (Figure 23,24). The highest, lowest and average values of (BIC%) obtained from micro-CT and comparing for statistical differences between groups are given in Tables 4.5 and 4.6 (Figure 25).

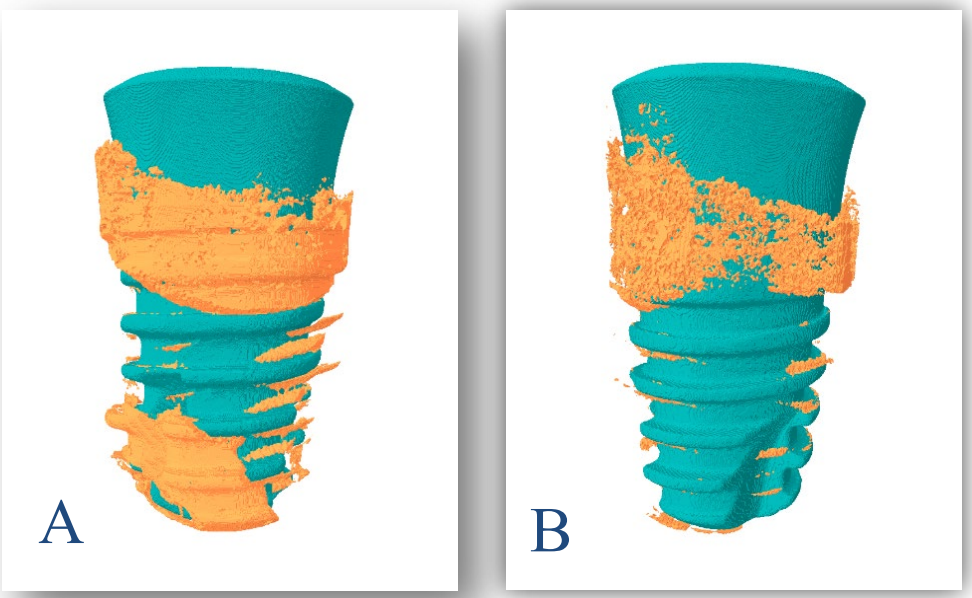


Figure 23. Micro CT images showing three-dimensional bone-implant contact in the control group (A) and OVX group(B).

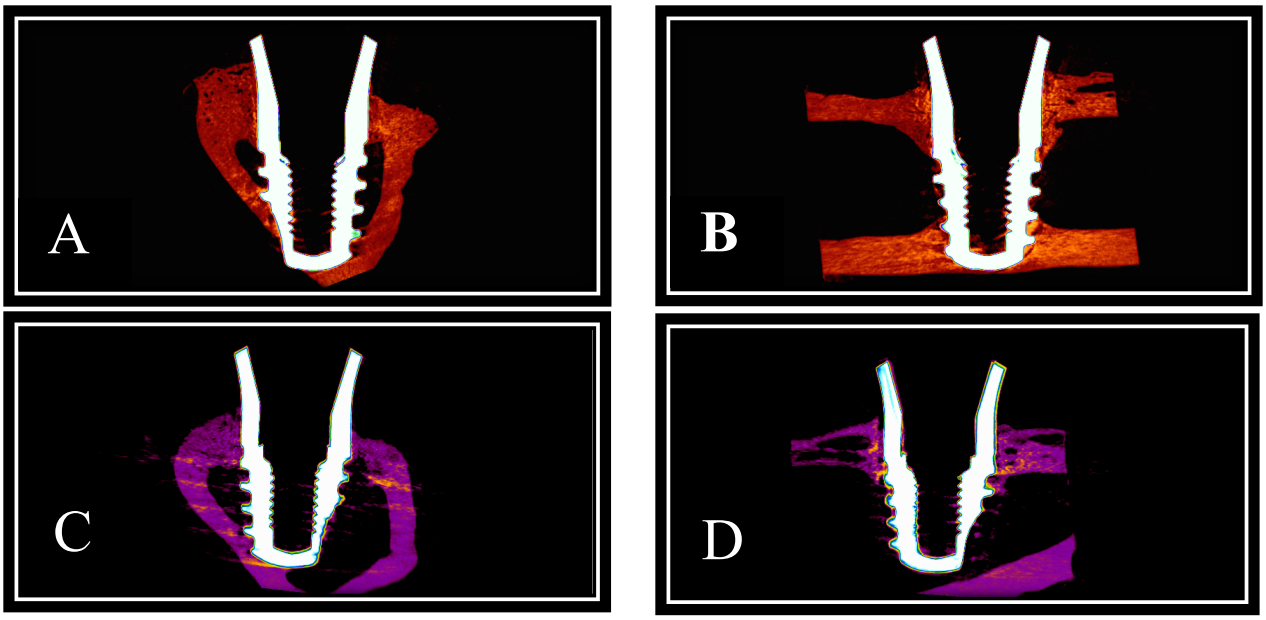


Figure 24. Coronal and sagittal sections of bone-implant contact in the control group (A, B) and OVX group (C, D) (two-dimensional micro-CT images)

Table 4.5. Highest, lowest and mean values of bone-implant contact percentage (BIC)

Groups	Highest BIC% Value	Lowest BIC% Value	Average BIC % Value ± Standard Deviation (SS) (N)
Group 1 (Control)	48.03	30.06	40.7±6.8 (10)
Group 2 (OVX)	28.64	18.15	24.14±3.5 (8)
Group 3 (Combined)	52.97	20.1	41.1±10.06 (8)
Group 4 (Sequential)	37.74	20.77	28.5±4.5 (9)
Group 5 (PTH)	43.08	18.01	32.2±6.9 (9)
Group 6 (Ral)	40.9	24.34	32.05±5.4 (9)

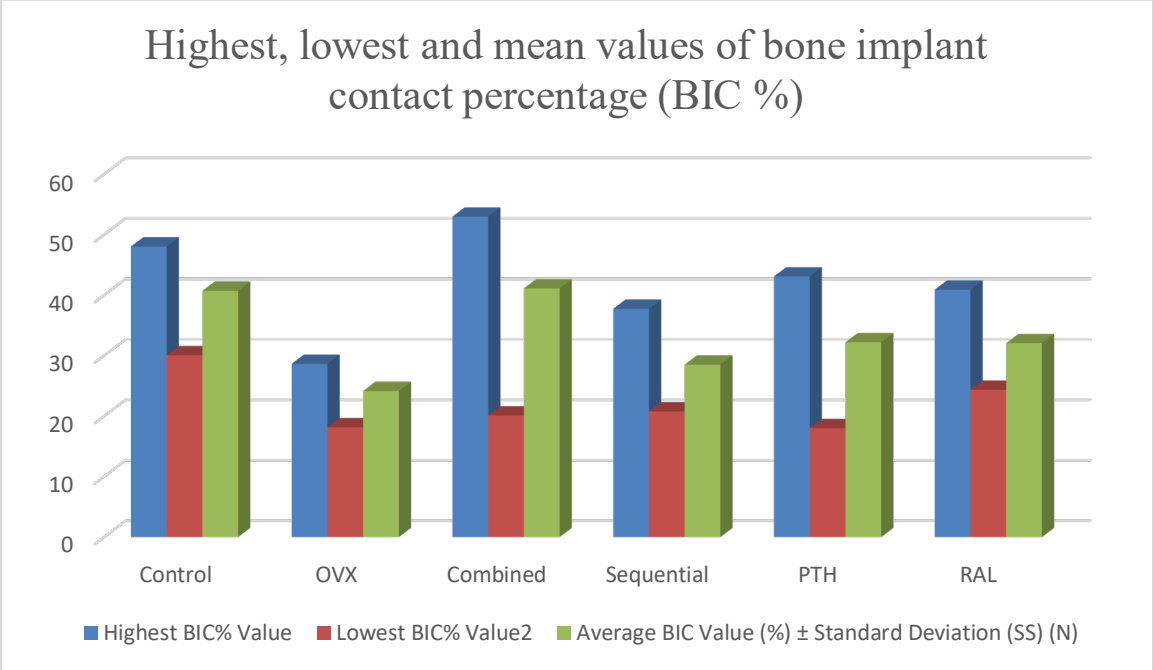


Figure 25. Highest, lowest and mean values of bone-implant contact percentage (BIC %) of experimental groups

Table 4.2. Percentage of bone-implant contact between groups (BIC%) group comparing statistical differences

	Difference Between Averages BIC%	**p value**
Group 1 (Control)-Group 2 (OVX)	16.63	***<0,001****
Group 3 (Combined)-Group 1 (Control)	0.3	1
Group 1 (Control)-Group 4 (Sequential)	12.2	***0.002***
Group 1 (Control)-Group 5 (PTH)	8.5	0.07
Group 1 (Control)-Group 6 (Raloxifene)	8.7	0.06

Group 3 (Combined)-Group 2 (OVX)	16.9	***<0,001****
Group 4 (Sequential)Group 2 (OVX)	4.4	0.7
Group 5 (PTH)-Group 2 (OVX)	8.1	0.1
Group 6 (Ral)-Group 2 (OVX)	7.9	0.1
Group 3 (Combined)-Group 4 (Sequential)	12.5	***0.003***
Group 3 (Combined)-Group 5 (PTH)	8.85	0.07
Group 3 (Combined)-Group 6 (Ral)	9.05	0.06
Group 4 (Sequential)-Group 5 (PTH)	3.7	0.8
Group 6 (Ral)-Group 4 (Sequential)	3.5	0.8
Group 6 (Ral)-Group 5 (PTH)	0.2	1

4.3.1. Micro-CT Morphometric Findings

Bone surface density (BS / TV mm^{-1}), percentage of newly formed bone volume (BV / TV%), percentage of total porous area Po (tot) %, Trabecular (Tb.Th Thickness) and percentage of bone contact surface area of implant I.S. / T.S. % were all measured in Micro-CT scan examination. Analysis of variance one-way ANOVA test was used for statistical evaluation because data followed a normal distribution.

Statistical differences between study groups and the highest, lowest and mean values are given in Table 4.7 and 4.8.

4.3.2. Percentage of bone volume (BV/TV%) findings

The mean (BV/TV%) values were obtained from all groups for statistical differences. The control group was compared separately with each of OVX, sequential, PTH and RAL group

and was shown to be statistically significant ($p < 0.001$) (Table 4.7). The mean (BV / TV%) value that was obtained from the control group (44.4 ± 10.6) shows a higher value than the mean values of the other groups. The mean (BV / TV %) values that were obtained from the OVX Group (17.3 ± 5.2) was found to be lower than of all other groups. The mean value of the combined group approached the control group's value and the difference was not statistically significant (32.1±13.1) ($p > 0.05$). Statistical differences between groups and the highest, lowest and mean values of (BV/TV%) findings are given in Table 4.8.

4.3.3. Bone Surface Density Findings (BS / TS mm^{-1})

The mean (BS / TS mm^{-1}) value was obtained from all groups for statistical differences. The control group was compared separately with each of OVX, sequential, PTH and raloxifene group and it was shown to be statistically significant ($p < 0.001$, $p < 0.05$, $p < 0.05$ and $p < 0.05$) respectively (Table 4.7). Results that were obtained from the control group (11.2 ± 2.1) showed a higher value than the other groups. The mean (BS / TS mm^{-1}) values obtained from the OVX group (6.2 ± 1.3) were found to be the lowest and the reason for this was thought to be the decrease in bone quality and density due to osteoporosis. The mean value of the combined group (8.7 ± 1.7) was found to approach the control group and the difference was not statistically significant ($p > 0.05$). Statistical differences between groups and the highest, lowest and mean values of (BS / TS mm^{-1}) findings are given in Table 4.7 and Table 4.8.

4.3.4. Percentage of Total porous Area Po (tot) %

The mean Po (tot) % value was obtained from all groups for statistical differences. The control group was compared separately with OVX, sequential, PTH, raloxifene and the combined group and it was shown to be statistically significant ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.05$ and $p < 0.05$, respectively) (Table 4.7).The mean Po (tot%) value

that was obtained from the control group (54.02 ± 10.5) show to be lowest than all other groups. The mean Po (tot) % value that was obtained in the OVX group (82.3 ± 5.3) was found to be highest, and the reason for this was thought to be the decrease in bone quality and density due to osteoporosis. Although the mean value of the combined group (68.8 ± 13.2) approached the control group, the difference was found to be statistically significant ($p<0.05$). Statistical differences between groups and the highest, lowest and mean values of Po (tot) % findings are given in Table 4.7 and Table 4.8.

4.3.5. Trabecular Thickness Findings (Tb.Th μm)

The mean value (Tb.Th μm) data was obtained from all groups for statistical differences. The control group was compared separately with OVX, sequential, PTH and the raloxifene group ($p<0.05$,$p <0.001$, $p<0,05$ and $p <0.001$, respectively) and also the combined group was compared separately with each of OVX and sequential group ($p <0.05$) (Table 4.7). The mean value of (Tb.Th μm) obtained from the control group (0.16 ± 0.014) was shown to be higher. The mean value of (Tb.Th μm) obtained from the raloxifene group (0.11 ± 0.01) was found to be lowest and the reason for this was thought to be the decrease in bone quality and density due to osteoporosis. The mean value of the combined group (68.8 ± 13.2) approached the control group, but the difference between them was not statistically significant ($p> 0.05$). Statistical differences between groups and the highest, lowest and mean values of (Tb.Th μm) findings are given in Table 4.7 and Table 4.8

Table 4.7: Average of the micro-CT parameters by groups. Mean, standard deviation (SD) and number of subjects (n).

	Control group [AVG±SD (n)]	OVX group [AVG±SD (n)]	Combined group [AVG±SD (n)]	Sequential group [AVG±SD (n)]	PTH group [AVG±SD (n)]	RAL group [AVG±SD (n)]
Bone Volume Percentage (BV/TV%)	44.4±10.6(10)	17.3±5.2(8)	32.1±13.1(8)	20.8±9.5(9)	22.8±6.6(9)	18.6±3.8(9)
Bone Surface Density (BS/TS $mm^{-1)}$	11.2±2.1(10)	6.2±1.3(8)	8.7±1.7(8)	8.1±2.4(9)	7.7±2.06(9)	7.7±1.2(9)
Percentage of Total Porose Area (Po(tot)%)	54.02±10.5(10)	82.3±5.3(8)	68.8±13.2(8)	78.7±9.5(9)	75.5±6.8(9)	80.2±4.1(9)
Trabecular (Tb.Th um)	0.16±0.014(10)	0.117±0.02(8)	0.15±0.02(8)	0.116±0.02(9)	0.12±0.02(9)	0.11±0.01(9)

Table 4.8: Statistical analysis of the differences between the mean values of micro-CT parameters between groups.

	Percent of Bone Volume (BV/TV%)	**Bone Surface Density (BS/TV $mm^{-1)}$**	**Total Porosity Percent (Po(tot)%)**	**Trabecular (Tb.Th Thickness)**
Control - OVX	27.04^{**}	5.1^{**}	-28.3^{**}	0.04^{*}
Control - Combined	12.2	2.5	-14.8^{*}	0.08
Control - Sequential	23.5^{**}	3.1^{*}	-24.7^{**}	0.04^{**}
Control - PTH	21.5^{**}	3.5^{*}	-21.4^{**}	0.03^{*}
Control - Ral	25.8^{**}	3.5^{*}	-26.2^{**}	0.05^{**}
Combined - OVX	14.7^{*}	2.4	-13.5^{*}	0.03^{*}
Sequential - OVX	3.5	1.9	-3.6	0.006
PTH - OVX	5.4	1.5	-6.8	0.006

Ral - OVX	1.22	1.4	-2.1	0.007
Combined - Sequential	11.2	0.5	-9.8	0.03*
Combined - PTH	9.2	0.9	-6.6	0.02
Combined - Ral	13.5	0.9	-11.4	0.04*
PTH - Sequential	1.9	0.4	3.2	0.006
Sequential - Ral	2.2	0.4	-1.5	0.006
PTH - Ral	4.2	0.02	-4.7	0.01

* Statistical difference between groups, $p<0.05$

** Statistical difference between groups, $p<0.001$

4.4 Histomorphometric findings

In the statistical evaluation, the One-Way ANOVA test was performed due to the normal distribution of the data that was obtained from (BIC%). There was a statistical difference between the groups ($p=0.004$). The result has shown that the difference was statistically significant between all study groups ($p = 0.004$). When the mean BIC% values of the groups were examined, it was seen that the highest value was obtained from the combined group 51,2%, followed by the control group 48.9%. The lowest mean BIC% value was obtained from the OVX group (28.6%). Control with OVX ($p = 0.01$), Combined with OVX ($p = 0.006$) The difference between the groups was found to be statistically significant. The difference between OVX and the control group ($p = 0.01$), OVX and the combined group ($p = 0.006$) was found to be statistically significant. Statistical differences between groups and the highest, lowest and mean values findings are given in Table 4.9 (Figure 20).

Table 4.9: Mean values of (BIC%) obtained from histomorphometry analysis.

Average (Mean), standard deviation (SD) and the number of subjects (n).

* Statistical difference between groups, $p<0.05$

** Statistical difference between groups, $p<0.001$

	Control [Avg±SD (n)]	OVX [Avg±SD (n)]	Combine [Avg±SD (n)]	Sequential [Avg±SD (n)]	PTH [Avg±SD (n)]	RAL [Avg±SD (n)]
Bone-implant Contact (BIC%)	48.9± 12.06(10)	28.6± 7.7 (8)	51.2± 12.9(8)	35.8± 9.6(9)	42.5± 18.08(9)	44.2± 8.4(9)

Statistical differences between the values BIC%.

Groups	Difference (%)	p value
Group 1 (Control)-Group 2 (OVX)	20.3*	**0.01**
Group 3 (Combined)-Group 1 (Control)	2.2	0.99
Group 4 (Sequential)-Group 1 (Control)	13.08	0.19
Group 1 (Control)-Group 5 (PTH)	6.44	0.85
Group 6 (Ral)-Group 1 (Control)	4.6	0.95
Group 3 (Combined)-Group 2 (OVX)	22.5*	**0.006**
Group 4 (Sequential)- Group 2 (OVX)	7.2	0.81
Group 5 (PTH)-Group 2 (OVX)	13.9	0.18
Group 6 (Ral)-Group 2 (OVX)	15.6	0.09
Group 3 (Combined)-Group 4 (Sequential)	15.3	0.11
Group 3 (Combined)-Group 5 (PTH)	8.6	0.67
Group 3 (Combined)-Group 6 (Ral)	6.9	0.84
Group 4 (Sequential)-Group 5 (PTH)	6.63	0.85
Group 6 (Ral)-Group 4 (Sequential)	8.3	0.68
Group 6 (Ral)-Group 5 (PTH)	1.7	1

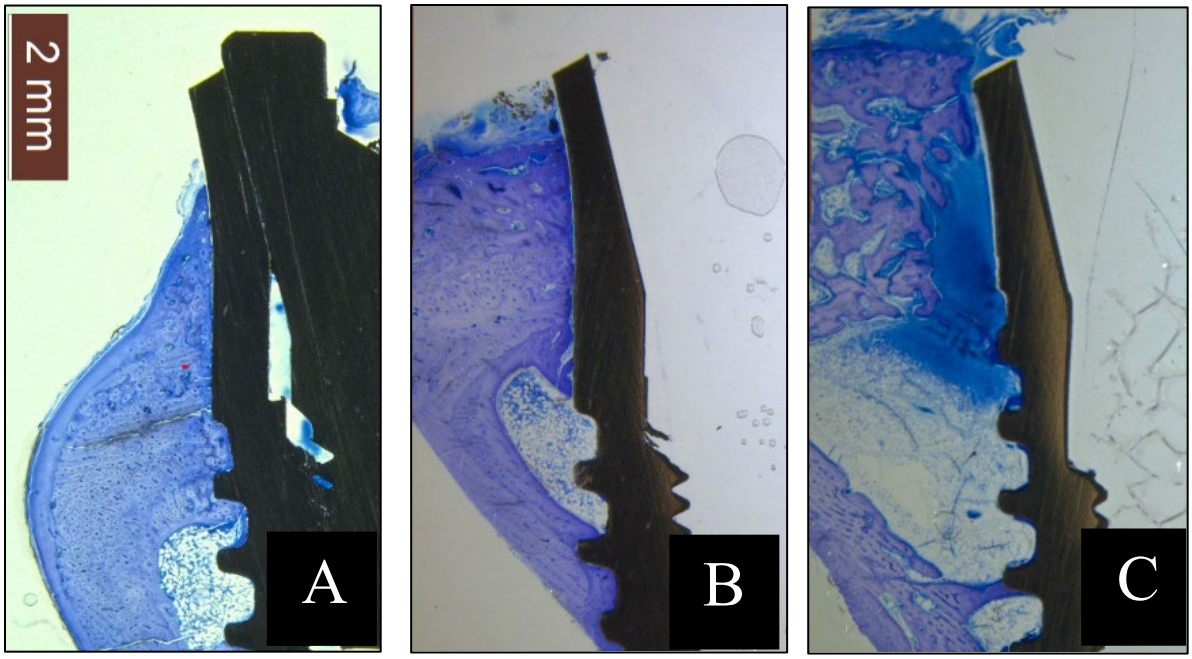


Figure 26: Ground section of the dental implants with the surrounding bone from the control (A), combined (B) and OVX (C) group, Bone is penetrating the area between the threads to the implant surface in the control group and combined group. Cortical bone porosity can be clearly seen in OVX (group toluidine blue staining, images magnification×10).

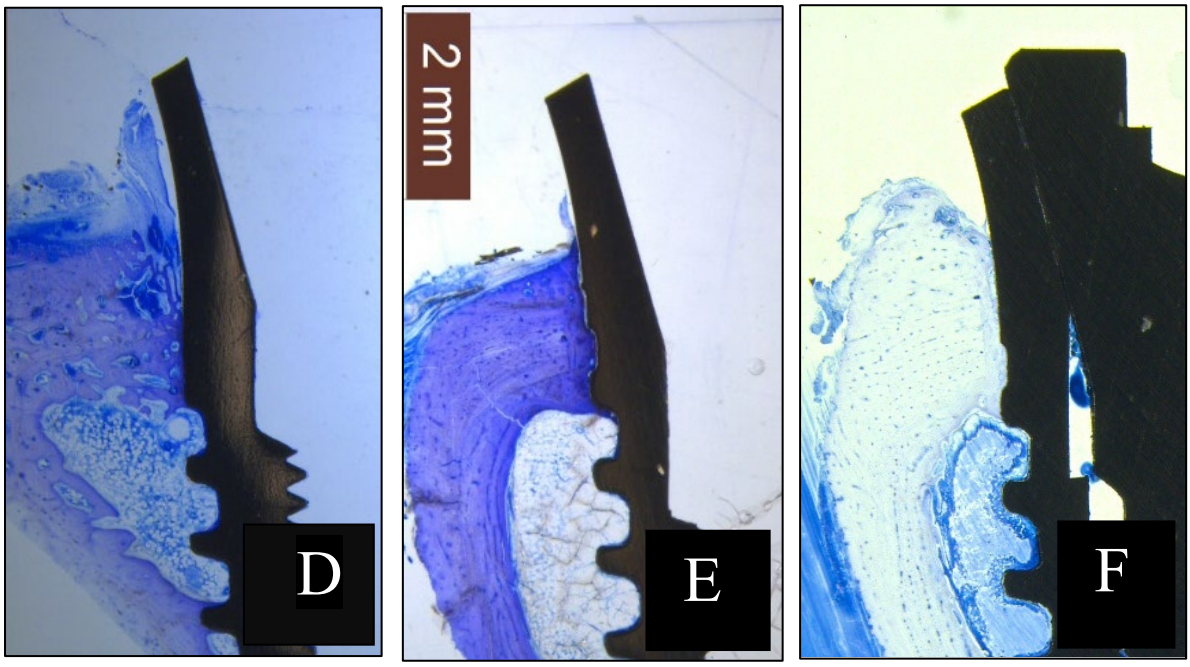


Figure 27: Ground section of dental implants with the surrounding bone taken from sequential (D), PTH (E) and RAL (F) (group toluidine blue, images magnification×10

5.DISCUSSION

Intra-bone dental implants are frequently used in prosthetic treatment in order to restore the esthetic and functional loss in a partial or in a completely edentulous patient. Dental implants have changed and developed over the centuries and have become one of the most indispensable treatment materials of today's dental practice.

Osseointegration plays an important role in the success of intra-bone dental implants and is described by Branemark as an uninterrupted structural and functional relation among vital bone and the surface of a load-carrying implant [123]. The long-term success of implant osseointegration depends on the correct examination of the area where the implant will be placed and careful planning [124]. Another important factor determining the outcome of the implant treatment is the quality of the bone surrounding the implant. An implant that is placed in bone with high density has less micro-mobility which will lead to a gradual stable improvement and a decrease in stress concentrations [124]. In the presence of dense bone, the percentage of implant-bone contact increases and implant stability improves after surgery during the recovery period [124]. One of the main reasons for implant failure and poor primary stability is the lack of bone density and decreased bone quality.

One of the diseases causing low bone quality and quantity is osteoporosis. Osteoporosis-related changes in the jaws are not different from other bones of the body [125] It is difficult to study osteoporosis because the disease is limited to humans only Osteoporosis is a gradually progressive disease that requires several years of follow up to see any kind of therapy result. The slow rate of the therapy results and the difficulty to maintain a study group are due to the natural attritions of the study groups. Bone density and quality are

adversely affected, so we can notice a decrease in aging cell proliferation, cellular synthesis activity, cellular susceptibility to local factors, and mesenchymal stem cell count. Due to these biological differences, the failure risk increases in the integration of any biomaterial in osteoporotic bone. For these reasons, it is important to improve the osseointegrations of biomaterials applied in osteoporotic bones and reduce the risk of unsuccessful osseointegrations. As a treatment option, various pharmaceutical agents are used to increase the osseointegration of dental implants in osteoporosis patients. These medications are categorized as antiresorptive agents; bisphosphonates providing osteoclast apoptosis, anti-receptor activator of nuclear factor-kappa B ligand antibody (Denosumab) that inhibit osteoclast uptake, receptor activator and selective estrogen receptor modulators (SERM), and an anabolic agent that can be used as teriparatide [126]. After the use of the antiresorptive medication, BMD can be only increased to a certain level by decreasing the number of osteoclasts and consequently the inability to synthesize new bone by osteoblasts (115). In those cases, it can be thought that the treatment protocol with antiresorptive drugs will limit the positive effects in the osseointegration process. But it has been reported that some antiresorptive drugs that are used may develop undesirable side effects in procedures like tooth extraction and intra-bone implant application (116). MRONJ (drug-induced jaw osteonecrosis), which develops especially after bisphosphonate use and dental procedures such as tooth extraction, seriously affects the patient's daily activities and quality of life.

Teriparatide is a synthetic polypeptide hormone that consists of 1-34 amino acid fragment of recombinant human parathyroid hormone (PTH 1-34). It is the first drug that was used for the anabolic treatment of diseases affecting the skeletal system [127]. Ogita et al. In their study examining the rat periosteal tissue and found that intermittent PTH initially induced periosteal osteoblast development, but also the continued administration suppressed cell proliferation [128]. Intermittent low doses of teriparatide have been reported to accelerate bone formation and provide a rapid increase in bone mass with improved microstructure. Teriparatide increases the mechanical resistance of bone in osteoporosis patients and reduces the risk of vertebral and non-vertebral bone fracture [129]. Teriparatide accelerates metabolic activity by indirectly regulating osteoblast functions and increases osteoclast numbers. These results were supported by experimental studies. In a study conducted on rats, Ma et al. reported that alendronate strongly

suppressed bone formation rate, and after 2 months of teriparatide treatment, mineral relocation and bone formation rate were increased. Periodic administration of teriparatide increases the biomechanical strength of the bone along with the amount of cortical and spongious bone.

Raloxifene is a selective estrogen-receptor modulator that binds to estrogen-receptors with an estrogen agonistic effects in some tissues and estrogen antagonistic effects in others. It is the first SERM that was approved to use on a patient with postmenopausal osteoporosis and was marketed for the prevention and the treatment of this disease. Giro G et al. and Luvizuto ER et al. reported better alveolar healing and also a greater histomorphometric result for the recently formed bone after estrogen or raloxifene therapy in osteoporotic rats [130, 131]. Estrogen ability to activate osteoblasts has been proved in former studies [130, 132]. The effect of raloxifene treatment on bone was reported in multiple studies and has shown to protect bone tissue and in activating mature osteoclasts and their survival [133]. Luvizuto ER et al. investigated bone healing in ovariectomized rats after using raloxifene and hormone replacement therapy and found that raloxifene balances out OVX statement by lowering the number of mature osteoclasts and pre-osteoclasts [134]. This information confirmed raloxifene therapy contribution in protecting bone tissue and keeping bone homeostasis [135-137] . The aim of this study was to evaluate the effects of raloxifene (SERM) and teriparatide on the osseointegration of dental implants.

There are many studies that evaluate the success of dental implants applied in experimentally generated osteoporosis-like situations with an experimental osteoporosis animal model [138] [139]. One of the reasons is that osteopenia that is developed in animals after ovariectomy is similar to humans' osteopenia [140]. Animal models provide a more homogeneous test material and allow them to perform wider analysis in many potential treatments. A well-selected experimental animal model that is suitable for the osteoporosis study minimizes the limitations of disease in humans and behavioral variability between tested subjects[141]. In this context, mammals such as rats, rabbits, mice, pigs can be used as an experimental animal. Rats are one of the most used animal models for osteoporosis studies because of their easy availability, rapid metabolism and fast generation time. People are familiar with the role of rodents for the usage in

experimental studies because they are low-cost and easy to house. In the literature, OVX rats are used extensively in histomorphometry and biochemical analyses [142]. The disadvantages of using a rat model in experimental researches are that sometimes estrogen deficiency occurs more than menopause, difficulties in repetitive blood and bone sampling, the intracortical havers canal system is not affected by the bone turnover, and in some regions, the bone turnover is faster than humans [114, 118]. Unlike other mammals such as rats, mice, and pigs, the usage of rabbit in research are found more beneficial because they reach skeletal maturity immediately after full sexual development, and their skeletal maturity is completed in as little as 5-6 months. On the other hand, the rabbit is used frequently in orthopedic studies due to its convenient bone size, easy supply and homogeneous breeds. The New Zealand White rabbits are normally used in experimental studies because they have a faster bone turnover than primates and a quicker developmental time[141]. They appear more common in the studies related to bone ingrowth after implant insertion. Establishing an experimental animal model of osteoporosis in rabbits can be very useful for investigating bone-acting anabolic agents, modeling processes, reconstruction rates as compared to other species [121]. In addition to all these features, rabbits are adapted as an experimental model because there are more easily obtained which makes him a suitable model for osteoporosis studies [121]. Another reason for choosing the rabbit model is that the dental implant sizes are quite large for the rat skeleton. In the literature, approximately 35 % rabbit model is used in musculoskeletal studies[143]. In the light of this information, the rabbit model was used in our study and intramuscular steroid (1mg / kg) was administered daily after the ovariectomy procedure to induce osteoporosis. In the present study, dental implants of 4.1 mm in diameter and 6 mm in length were applied in a similar manner with reference to other studies that were applied to the rabbit tibia [144, 145]. These implants are specially manufactured for the use in our research. The placement of dental implants has been performed by a single operator, in order to achieve standardization.

Cao et al. reported that BMD decreased significantly in the mandible of an ovariectomized rabbit after 12 weeks[146]. It has been reported that osteoporotic deterioration has started in ovariectomized rabbit femur starting from 2 months and osteoporosis can be observed significantly at 4 months[147]. In our study, we waited 8 weeks after the ovariectomy

procedure, and the intramuscular steroid administration was performed for a period of 4 weeks during the waiting period. After that period implant surgery was performed.

In the present study, similar studies in the literature have been taken into consideration in determining the duration of implant healing. In the literature, although the number of studies in which an osteoporotic rabbit model was implanted to the tibia bone is limited, the expected duration of osseointegration, in general, varies between 6 and 12 weeks [148, 149].In light of this information, it was thought that the 12-week period may be sufficient to see the effect of osteoporosis on the osseointegration of implants placed in the rabbit tibia. In the studies carried out by Mori et al. and Lugero et al, at the end of an 8-week osseointegration period, implant integration in the osteoporotic group was significantly lower than implant integration of the control group and that result supports the waiting period in our study [148, 149].

The evaluation of implant osseointegration was performed by histomorphometric, densitometric and biomechanical test analyzes methods. Dental implant stability is achieved by successful osseointegration and is deemed critical for implant stability, is considered a prerequisite for implant loading and long-term clinical success of endosseous dental implants. Therefore, measurement of implant stability is an important method to evaluate the success of osseointegration [150]. There are some biomechanical test methods used to evaluate the implant's stability. For the evaluation of implant stability, Periotest, Resonance Frequency Analysis (RFA), removal torque tests, percussion test, and Implatest can be used [151]. RFA method was first used in 1996 that measures the stability of the implant without damaging the implant and thus provides an idea of the state of osseointegration. It is also a noninvasive method for uninterruptedly evaluating implant stability in clinical cases. The measurement is carried out with the help of a spacer called a transducer (SmartPeg) screwed to the implant with its magnet on top, which 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. The device measures the frequency of the resonance occurring at the interface and reflects it on the screen and reports a value between 0-100. This value obtained as a result of the measurement is called

ISQ (implant stability quotient) [121]. These data verify the bone volume surrounding the implant and whether the bone and implant surfaces have integrated or not. Among the studies, a strong correlation was found between the histomorphometric and RFA findings during the osseointegration stage [151]. In our study, RFA was performed to evaluate both primary stability (T0) and secondary stability (T1). Primary stability is defined as the mechanical adaptation and mobility absence among the implant surface and bone immediately after implant placement. Secondary stability are related to bone healing progression around a dental implant, and it's established after primary stability and also after implant osseointegration that was gained from bone regeneration and remodeling [152, 153]. According to T0 results of the RFA analysis, the data of the control group was found to be statistically higher than other groups. No statistically significant difference was found in T0 period from osteoporosis study groups. This result was thought to be due to the decrease in BMD after osteoporosis that was initiated by ovariectomy and glucocorticoid administration before implant surgery. In the T1 period, the mean value of the OVX group was the lowest and the differences between the other groups were found to be statistically significant. However, the difference between the groups was not statistically significant. According to the results, medical therapies contribute to osseointegration of dental implants. In previous experimental studies by Que H et al. and Yıldız A et al., it was reported that implants applied to osteoporotic bone give lower results in the stability tests [121, 154]. Oki Y et al. examined the influence of parathyroid hormone (PTH) on the primary stability of dental implants in a osteoporotic model. According to the results, ISQ value in the PTH-group was much higher than the ISQ value of the OVX-group (74.7 ± 11.2 and 55.9 ± 13.5, respectively; $P < 0.05$) . Similar to our study they used ovariectomy and glucocorticoid administration by combining the effect of both treatments to form an osteoporosis model using rabbits. Also in a study by Castaneda S et al. rabbit model was used to measure bone mineral content and BMD using a dual energy X-ray, the result showed that BMD was significantly decreased after the ovariectomy and glucocorticoid administration [113] .The data obtained in this study is in accordance with the results of previous studies.

Another biomechanical test used in the study is the removal torque. Removal torque has been recognized by many researchers to provide reliable results when good standardization

is achieved [155]. Removal torque is applied to removes the implant from the implant screw bore. This test is particularly preferred in animal studies. In most cases, this test usually includes digital torque gauges and force gauges such as dynamometers. In this study, measurements were performed manually with digital torque meter on standardized test apparatus. Peng et al reported that implant removal torque should be performed soon after animal sacrification to prevent bone dehydration that could interfere with the result by changing physiologic and mechanical properties [156]. In literature studies, it was seen that removal torque mean values of implants that were applied in osteoporosis bone model were lower than removal torque values of the implant that was applied in healthy groups [121, 157-159]. The removal torque data of this study are generally in line with the literature. However, the values in osteoporosis groups given medication (all groups except control and OVX) exceeded the values of the control group but did not show statistically significant differences. Value differences between the combined group and the OVX group were found to be statistically significant. In the light of these results, removal torque data showed that medication increased the implant osseointegration and removal torque values from the groups that were given medication were found to close to control group values despite osteoporosis. An increase in bone density around titanium dental implants of the ovariectomized rabbit could explain the better removal torque values in groups that received the medication. The improvement in the removal torque values observed in the test groups (all group except control and OVX) demonstrated that the administration of teriparatide and raloxifene did not act unfavorably on the process of osseointegration. However, no improvement in the removal torque could be observed in the statistical analysis between the intragroup analyses of the test groups.

In a study performed by Fujimoto et al., the mean removal torque value of the implants (45.8 ± 15.2 Ncm) that was placed in the tibial bone of osteoporotic rabbits was shown to be lower compared to healthy group (62.7 ± 14.9 Ncm). Jung C-Y et al. also measured the mean removal torque value of implants that were applied in the osteoporotic tibial bones of the rabbit model in each of osteoporosis and healthy group and the result was 35.6 ± 3.6 Ncm and 48.5 ± 5.4 Ncm, respectively. Finally, Wen B. et al. evaluated the osseointegrations of the implants coated with titanium-zirconium (Ti-Zr) and only titanium (Ti) by applying them to the tibial bones of osteoporotic and healthy rabbits. In the healthy

group, Ti-Zr implants removal torque mean value was 70.2 Ncm and in Ti implants was 45.7 Ncm. In the osteoporosis group, the mean of the removal torque value of Ti-Zr coated implants was 57.2 Ncm and that of Ti-coated implants was 37.8 Ncm. In our study, the mean removal torque value of implants in the control group was found to be 76.2±19.6 Ncm. In the OVX group, the mean value of removal torque was 49.6±12.5 Ncm. Although these results were close to the study of Wen B. et al, they were higher than the value of the other studies. We think that the reason for this is related to implant brand used, also to the design and the surface properties of the implant. At the same time, the groove structures of the implants used in our study are also are more aggressive than the other implants that were used in the literature. In addition, the average removal torque values of implants that were taken from the test group can be considered to increase due to the positive effects of the treatment. It can be noticed that the result of our study is on the same parallel and in consistent with the results of previous studies.

Osseointegration between bone and implants has been frequently evaluated in the literature by histomorphometric analysis [160]. However, histomorphometric is a destructive method and the same sample cannot be used to evaluate other tests such as removal torque measurement, stability assessment [160]. Especially in regenerative therapies, the data obtained in histomorphometric analyses used to evaluate new bone formation that are limited to two-dimensional images [161]. Another disadvantage of histomorphometric analysis is that only a few sections for each implant can be obtained by trimming methods [162]. In addition, the process of achieving the analysis is quite long.

There are various studies that used histomorphometric methods for the evaluation of implant osseointegration in experimental animal model. Yıldız A et al. studied the effects of systemic zoledronic acid administration on the osseointegration dental implants in a osteoporotic rabbit model. To performed histomorphometric analysis the bone to implant contact percentage was measured, the mean BIC% value was 53.01% in the control group, 43.08% in the experimental group, and 36.02% in the osteoporosis (OVX) group[121]. The result of this study showed that the higher value of BIC was obtained from the combined group and that histomorphometric data demonstrate the negative effects of ovariectomy on the implant to bone contact. In addition to this study, Faverani LP et al. evaluated the effect

of raloxifene and alendronate to compensate for the impaired osseointegration in osteoporotic rats. Histomorphometric data indicated a higher bone-to-implant contact with raloxifene but not with alendronate compared to the osteoporosis group. Raloxifene group value was close to the control group but didn't surpass it [163]. In our study, it was seen that the highest value was obtained from the combined group (51.2± 12.9%), followed by the control group (48.9%). The lowest mean BIC% value was obtained from OVX group (28.6± 7.7%). These results are in consistent with the literature (Yıldız A et al. and Faverani LP et al). According to this data obtained from our study, it has been seen that combine drug administration to the experimental animals has positive effects on dental implant osseointegration.

Micro-CT is accepted as the gold standard for the evaluation of trabecular microstructure, but it is not yet used clinically [164]. The pixels that make up 2 or 3-dimensional cross-sectional images obtained by micro-CT allow micro-dimensional visualization of the internal structure of a material in three dimensions without any nondestructive measurements. In addition, micro-CT use both live and different properties of solid or liquid samples effectively to examine [165]. In particular, it is known that micro-CTs are used in important subjects such as imaging soft tissue and bone tissues, examination of composite materials, metals and alloys [165]. In our study, Micro-CT and histomorphometric analyses were performed to determine bone quality and density and to examine the contact between implant and bone. Osseointegration between bone implants has been frequently evaluated in the literature only by histomorphometric analysis. In this study, additional to histomorphometric analysis, micro-CT was also used to evaluate the osteointegration. A 2-D and 3-D examination were performed by micro-CT.

Similar to literature, in our study some parameters such as percentage of bone-implant contact (BIC), percentage of bone volume (BV / TV%), density of bone surface (BS / TV), percentage of total porous area (% Po (tot)), Trabecular Thickness parameters (Tb.Th) were examined by micro-CT [166-168]. In the results of our study, contribution to implant osseointegration can be observed in test groups. These contributions also supported the values of the bone-implant contact obtained from the histomorphometric analyzes. The values of bone-implant contact showed a moderate, positive and statistically significant

correlation between the values obtained from micro-CT and the histomorphometric analysis. Combined therapy was found to be the most effective method when compared to the other methods. The data of the micro-CT morphometric analysis, histomorphometric bone-implant contact and biomechanical test results significantly was decreased in the OVX group. These results supported the development of osteoporosis in rabbits.

In a study with Ying Gao et al., 40 rats were divided into 4 groups and placed implants on the right and left tibial bones. They found that all parameters that was examined in the osteoporosis group (Tb. Sb, Tb. N, % BV / TV, BIC) decreased. In our study, it was observed that the same parameters were significantly decreased in the OVX group, similar to that in this study[168].

In a study by Mengchun Qi et al., dental implants were placed in the bone of ovariectomized rabbits. Three groups were formed; non-osteoporosis group, osteoporosis and osteoporosis with zoledronic acid (ZOL) administration. At the end of the study, % BV / TV, Tb.Th, Tb. Sb, BIC% values were examined in each group. According to their results, the mean BV / TV value of the control, osteoporosis and ZOL group was 56.17%, 23.19 and 54.68%, respectively. Also, the mean BIC percentage was found to be 62.92%, 30% and 55.11%, respectively[169]. In our study, % BV / TV values were found as 44.4±10.6% for the control group, 17.3±5.2% for OVX group, 32.1±13.1% for the combined group and 20.8±9.5 for the sequential group. These data were close to BV / TV% values obtained by Mengchun Qi et al. In addition, the decrease in BV / TV% value in the osteoporosis in this study compared to the healthy group was observed in our study. In this study, bone-implant contact mean value was found to be 40.7±6.8% for the control group 24.14±3.5 % for OVX group, 41.1±10.06 % for the combined group and 28.5±4.5 % for the sequential group. These data are in parallel to bone-implant contact values obtained by Mengchu et al. Also, the decrease of bone-implant contact value in OVX group in Mengchu et al. study was also observed in our study. The reason for the difference in values between Mengchu et al study and this study was thought to be related to the area scanned by micro-CT, used implant design, length and diameter, feeding and age of the rabbits.

Almagro et al. evaluated the effects of teriparatide on implant osseointegration by applying systemic teriparatide in an osteoporotic rabbit model [163]. Implants were placed in the

proximal tibia metaphysis of all animals. The healthy rabbit was used as a control group. Osteoporotic rabbits were divided into two groups that started saline vehicle or intermittent teriparatide administration for 12 weeks. After the sacrification process, histological and CT morphometric examinations were performed. The authors found that teriparatide significantly increased the BIC% of the dental implants in osteoporotic bones compared to osteoporotic and to healthy control groups. It was also indicated that teriparatide treatment may contribute to implant osseointegration in osteoporotic bones. On the other hand, according to the results of the same study, no difference was found between osteoporosis group and control group in terms of BIC%.

In another study done by Oki Y et al., the efficacy of teriparatide on implant osseointegration was evaluated in an ovariectomy-induced rabbit model using resonance frequency analysis and histological examinations [170]. Osteoporosis was induced by applying the same protocol used in our study and three group was formed. The first group received subcutaneous teriparatide before implant placement. The second group received subcutaneous teriparatide for 4 weeks before and 4 weeks after implant placement. The third group was used as the control group. According to the results, implant primary stability values were significantly higher in intermittent teriparatide groups compared to the control group. In addition, in the second and fourth-week ISQ values in the second group were significantly higher than both first and the third group. In histological evaluations, bone thickness and trabeculation were found to be higher in the second group compared to the first group, whereas the amount of bone around the implant was significantly higher in the second group compared to the first group and control group. The authors concluded that teriparatide treatment (in the presence of osteoporosis) had positive effects on dental implant stability and osseointegration. In this study, the authors started teriparatide application 4 weeks before the implant placement and ended 4 weeks after the implant placement, in contrast to teriparatide application methods in the literature.

In the literature, we can find studies that only studied the effect of drug administration on implant osteointegration without creating a model of osteoporosis. Corsini et al. evaluated the effect of intermittent administration of human parathyroid hormone on implant osseointegration using removal torque test [171]. Rabbits were divided into two groups as

experimental and control. The animals in the experimental group received teriparatide intermittently for 56 days, whereas the animals in the control group received placebo. According to the result of the study, removal torque values of the implants in the experimental group were found to be higher than the control group values, but this increase was not found to be statistically significant. However, in this study, the authors did not create any model of osteoporosis.

As in the studies mentioned above, in our study, an increase was observed in ISQ, retraction torque, histomorphometry, and micro CT values compared to the negative control group (OVX) when teriparatide was administered alone. However, this increase was not statistically significant.

One of the treatment modalities of osteoporosis is hormone replacement medication, however this therapy has some contraindications and side effects. Medication, containing selective estrogen receptor modulators (SERMs), are promising alternative treatments for osteoporosis. In the literature, it is possible to come across many studies investigating the effect of raloxifene on implant osseointegration in an ovariectomy-induced rabbit model. In a study by Heo HA et al, implants were placed in the upper jaws of osteoporotic rats and were divided into 3 groups: ovariectomized group (OVX), ovariectomized and raloxifene-administered group (RAL) and the control group. The OVX and the control group didn't take any medication therapy while the RAL group was administered with raloxifene. In each group, three rats were sacrificed after a specific time for radiologic and histologic evaluations. According to the results of the study, the mature bone formation around the implants in the RAL group was faster than the OVX group [172]. However, in this study, the authors did not perform any statistical analysis when evaluating the formation of new bone, but only evaluated the bone maturation around the implant on histological and micro-CT images.

Ramalho-Ferreira et al also investigated the efficacy of raloxifene on an osteoporotic rat model. The control group was considered as the healthy group. Three groups was formed from OVX rats: the first group didn't receive any medication the other two took either raloxifene or alendronate. In order to evaluate the osteointegration, they performed removal torque test and histomorphometric (BIC%) analyzes. In the raloxifene group, significant

results were obtained in terms of biomechanical and BIC% values, while there was no significant difference between the alendronate group and the OVX group [173]. In the same study, the authors stated that the values between the raloxifene group and the healthy group were not statistically significant.

In our study, although the biomechanical and histomorphometric values of the raloxifene group were increased compared to the OVX group, this increase was not statistically significant parallel to the literature.

There are several studies in the literature investigating the sequential and combined therapy strategy. In sequential therapy, adding antiresorptive drug to on-going teriparatide treatment generally have a good result. Non-experimental studies indicate that BMD values decrease rapidly in patients who do not take antiresorptive medication after ending teriparatide treatment, whereas antiresorptive agent administration after teriparatide treatment can preserve or improve teriparatide-induced BMD gain further [174-177]. In an experimental study in which teriparatide and raloxifene were applied sequentially, the possible effects of sequential administration of alendronate, raloxifene and teriparatide on the collagen and osteoporotic bones strength in ovariectomized rabbits were investigated [178]. In this study, the authors administered teriparatide for five months in osteoporotic rabbits then divided into two groups and given raloxifene or alendronate for 5 months. They also formed a negative control group that underwent only ovariectomy. After the sacrification procedure, BMD was measured, and biomechanical test was also performed. Results showed that BMD and strength of osteoporotic bones treated with raloxifene and alendronate after teriparatide increased compared to the control group and emphasized that sequential treatment may be effective in the treatment of osteoporosis. In our study, the data obtained from micro-CT analyses showed that bone mineral density increased compared to OVX group and successive treatment resulted in an increase in trabecular thickness and new bone volume. However, this increase was statistically significant only in trabecular thickness.

In addition to sequential treatment, we can see that in the literature multiple studies proved that combined use of an anabolic and antiresorptive agent have also better result in increasing bone quality and strength after osteoporosis. In the study of Cosman et al., the

effect of combination therapy on BMD and bone turnover markers was examined in postmenopausal women with osteoporosis [179]. Patients were randomized to a single dose of zoledronic acid plus daily teriparatide, only teriparatide, or zoledronic acid. Spine BMD increased faster in the group that was given combination therapy than with either treatment alone. Also, total hip BMD increased more in the combination group than in monotherapy group. Chad et al. studied the effect of the combined administration of teriparatide and raloxifene in a double-blind placebo-controlled trial. The author evaluated the combination treatment with teriparatide and raloxifene (first group) with teriparatide alone (second group) in women with postmenopausal osteoporosis. Bone building-destruction markers and bone mineral densities of patients treated was measured [7]. In the study, bone resorption and formation were evaluated by serum markers (CTx, PINP) at the end of six months. Bone mineral density was also measured by DEXA test. According to the results, bone formation values obtained from the first group were found to be similar to the values of the second group, while bone resorption values in the first group was significantly lower than the second group. In addition, BMD values in combined therapy were significantly higher than the value of the teriparatide-only group. The authors emphasized that the combined therapy with raloxifene may increase the bone-forming effects of teriparatide.

In our study, the data obtained from the micro-CT analyses showed that BMD increased when comparing the value between the OVX group and the combined group. In addition, trabecular thickness and new bone volume of the combined group increased statistically.

While there are experimental studies investigating the effects of raloxifene and teriparatide on implant osseointegration when administered alone, no studies investigating the effects of these drugs on osseointegration of dental implants when applied sequentially or in combination. In the present study, when these drugs were administered separately, they contributed positively to implant osseointegration and bone formation in osteoporotic bones. On the other hand, there are a limited number of experimental and clinical studies investigating the efficacy of these drugs on osteoporotic bones when administered sequentially or in combination. Those studies have shown that sequential and / or combined teriparatide and raloxifene treatments increase the density and strength of osteoporotic bones. The main hypothesis of the combined usage of these two drugs is the preservation

and/or enhancement of the new bone formation. In the present study, our results supported this hypothesis and it was observed that osseointegration and bone formation around the implant increased significantly especially after combined therapy (teriparatide and raloxifene). In healthy bones, the bone remodeling process carries a continuous and balanced formation and resorption mechanism, however, in the case of osteoporosis, the balance of this resorption and formation mechanism is in favor of resorption. In this study, one of the main reasons for achieving more successful results in combined therapy compared to single or sequential administration of these drugs can be explained as rebalancing of the bone remodeling mechanism in osteoporotic bones by increasing bone production while simultaneously slowing bone destruction.

6.Results

-When teriparatide and raloxifene were used individually, the treatment contributes to dental implant osseointegration in osteoporotic bones positively, but the results were not statistically significant.

-The sequential use of teriparatide and raloxifene contributed more than the individual usage of these drugs on dental implants osseointegration that was placed in osteoporotic bones, but this improvement was not statistically significant.

- The combined use of teriparatide and raloxifene contributes significantly to the osseointegration of dental implants that were placed in osteoporotic bones when compared to single and sequential administration of these drugs.

- In light of all these results, it was concluded that the most effective pharmaceutical method to increase the success of dental implants applied in low-density bones is the combined use of teriparatide and raloxifene.

- However, the results obtained from our study should be supported by further experimental and clinical studies.

Uncategorized References

1. Pilliar, R., et al., *Observations on the effect of movement on bone ingrowth into porous-surfaced implants.* 1986(208): p. 108-113.
2. Kurland, E.S., et al., *Parathyroid hormone as a therapy for idiopathic osteoporosis in men: effects on bone mineral density and bone markers.* 2000. **85**(9): p. 3069-3076.
3. Beikler, T., T.F.J.C.R.i.O.B. Flemmig, and Medicine, *Implants in the medically compromised patient.* 2003. **14**(4): p. 305-316.
4. Eriksen, E.F.J.E.r., *Normal and pathological remodeling of human trabecular bone: three dimensional reconstruction of the remodeling sequence in normals and in metabolic bone disease.* 1986. **7**(4): p. 379-408.
5. McCormick, R.K.J.A.M.R., *Osteoporosis: integrating biomarkers and other diagnostic correlates into the management of bone fragility.* 2007. **12**(2): p. 113.
6. Harsløf, T. and B.L.J.C.o.i.p. Langdahl, *New horizons in osteoporosis therapies.* 2016. **28**: p. 38-42.
7. Deal, C., et al., *Combination teriparatide and raloxifene therapy for postmenopausal osteoporosis: results from a 6-month double-blind placebo-controlled trial.* 2005. **20**(11): p. 1905-1911.
8. İlhan, D., et al., *Dental İmplantlar ve İnfeksiyon.* Dental Tribune Türkiye, 2014. **4**: p. 7-9.
9. Adell, R., et al., *A 15-year study of osseointegrated implants in the treatment of the edentulous jaw.* International journal of oral surgery, 1981. **10**(6): p. 387-416.
10. Berenson, J.R., et al., *American Society of Clinical Oncology clinical practice guidelines: the role of bisphosphonates in multiple myeloma.* Journal of Clinical Oncology, 2002. **20**(17): p. 3719-3736.
11. Gotfredsen, K. and U. Karlsson, *A prospective 5-year study of fixed partial prostheses supported by implants with machined and TiO2-blasted surface.* Journal of Prosthodontics, 2001. **10**(1): p. 2-7.
12. Knabe, C. and K. Schendel, *The use of implant-supported titanium prostheses for treatment of periodontally compromised patients including functional and orthodontic therapy. Report of 2 cases.* Clinical oral implants research, 1997. **8**(4): p. 332-338.
13. TUNCER, S.D.E. and H. ÇANKAYA, *DENTAL İMPLANT TEDAVİSİNİN PLANLAMASI VE TAKİBİNDE GÖRÜNTÜLEME YÖNTEMLERİNİN ÖNEMİ VE KARŞILAŞTIRILMASI.*

14. Lemons, J. and J. Natiella, *Biomaterials, biocompatibility, and peri-implant considerations.* Dental clinics of north america, 1986. **30**(1): p. 3-23.
15. Ikebe, K., et al., *Is old age a risk factor for dental implants?* Japanese Dental Science Review, 2009. **45**(1): p. 59-64.
16. Branemark, P.-I., *Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period.* Scand. J. Plast. Reconstr. Surg. Suppl., 1977. **16**.
17. Albrektsson, T., et al., *Osseointegrated titanium implants: requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man.* Acta Orthopaedica Scandinavica, 1981. **52**(2): p. 155-170.
18. Zarb, G.A. and T. Albrektsson, *Osseointegration: a requiem for the periodontal ligament.* Int J Periodontics Restorative Dent, 1991. **11**(1): p. 88-91.
19. Fritz, P.C., B. Ganss, and H.C. Tenenbaum, *Temporal Relationship Between Expression and synthesis of Bone Matrix Proteins During Osteogenesis.* Aging, Osteoporosis and Dental Implants. Chicago: Quintessence, 2002: p. 217-227.
20. Jokstad, A., *Osseointegration and dental implants*. 2009: John Wiley & Sons.
21. Davies, J.E., *Understanding peri-implant endosseous healing.* Journal of dental education, 2003. **67**(8): p. 932-949.
22. Davies, J., *Mechanisms of endosseous integration.* International Journal of Prosthodontics, 1998. **11**(5).
23. Laney, W., *State of the science on implant dentistry: Consensus conference proceedings.* Int J Oral Maxillofac Implants, 2007. **22**: p. 1-226.
24. Esposito, M., et al., *Biological factors contributing to failures of osseointegrated oral implants,(II). Etiopathogenesis.* European journal of oral sciences, 1998. **106**(3): p. 721-764.
25. Albrektsson, T. and G.A. Zarb, *Current interpretations of the osseointegrated response: clinical significance.* International Journal of Prosthodontics, 1993. **6**(2).
26. Mombelli, A. and N.P. Lang, *Clinical parameters for the evaluation of dental implants.* Periodontology 2000, 1994. **4**(1): p. 81-86.
27. Bornstein, M.M., N. Cionca, and A. Mombelli, *Systemic conditions and treatments as risks for implant therapy.* Int J Oral Maxillofac Implants, 2009. **24**(Suppl): p. 12-27.
28. Hwang, D. and H.-L. Wang, *Medical contraindications to implant therapy: part I: absolute contraindications.* Implant dentistry, 2006. **15**(4): p. 353-360.
29. Alsaadi, G., et al., *Impact of local and systemic factors on the incidence of oral implant failures, up to abutment connection.* Journal of clinical periodontology, 2007. **34**(7): p. 610-617.
30. Hwang, D. and H.-L. Wang, *Medical contraindications to implant therapy: Part II: Relative contraindications.* Implant dentistry, 2007. **16**(1): p. 13-23.
31. Van Steenberghe, D., et al., *Impact of systemic diseases and medication on osseointegration.* Periodontology 2000, 2003. **33**(1): p. 163-171.
32. Zarb GA, L.U., Albrektsson T, Tenenbaum H,, *Aging, osteoporosis and dental implants.*, ed. L.U. Zarb GA, Albrektsson T, Tenenbaum H,. 2002: Quintessence. 229-241.

33. Albrektsson, T., et al., *The long-term efficacy of currently used dental implants: a review and proposed criteria of success.* 1986. **1**(1): p. 11-25.
34. Tardieu, P.B., et al., *Computer-assisted implant placement. A case report: treatment of the mandible.* 2003. **18**(4).
35. Ludlow, J.B., et al., *Dosimetry of 3 CBCT devices for oral and maxillofacial radiology: CB Mercuray, NewTom 3G and i-CAT.* 2006. **35**(4): p. 219-226.
36. Scarfe, W.C. and A.G.J.D.C.o.N.A. Farman, *What is cone-beam CT and how does it work?* 2008. **52**(4): p. 707-730.
37. Brägger, U.J.P., *Use of radiographs in evaluating success, stability and failure in implant dentistry.* 1998. **17**(1): p. 77-88.
38. Feldkamp, L.A., et al., *The direct examination of three-dimensional bone architecture in vitro by computed tomography.* 1989. **4**(1): p. 3-11.
39. Dario, L.J., P.J. Cucchiaro, and A.J.J.T.J.o.t.A.D.A. Deluzio, *Electronic monitoring of dental implant osseointegration.* 2002. **133**(4): p. 483-490.
40. Aparicio, C., N.P. Lang, and B.J.C.o.i.r. Rangert, *Validity and clinical significance of biomechanical testing of implant/bone interface.* 2006. **17**(S2): p. 2-7.
41. Geçkili, O., *İki farklı İmplant yüzeyinin kemik içi Stabilite ve Krestal kemik kayıpları üzerine etkilerinin; iki farklı Abutment tipinin hasta memnuniyetlerinin karşılaştırılması/Onur Genkili; dnş. Tayfun Bilgin.*
42. U., A., *Implant Tedavisi Uygulanacak Bolgelerin Tomografik Ve Histolojik Kemik Kalitesi Degerlendirilmesinin Implant Stabilitesi Ile Korelasyonu.*
43. ÇİNKILIÇ, Ö.S., *İyilesme Doneminde Uygulanan LED Fotobiyomodulasyon Tedavisinin Farklı Yuzey Ozelliklerine Sahip Dental Implantlarda, Implant Stabilite Oranlarına Etkisinin Rezonans Frekans Analizi Ile Karsılastırmalı Olarak Incelenmesi.* . 2011, Hacettepe Universitesi Saglık
44. Meredith, N., D. Alleyne, and P.J.C.o.i.r. Cawley, *Quantitative determination of the stability of the implant-tissue interface using resonance frequency analysis.* 1996. **7**(3): p. 261-267.
45. Östman, P.O., et al., *Direct implant loading in the edentulous maxilla using a bone density-adapted surgical protocol and primary implant stability criteria for inclusion.* 2005. **7**: p. s60-s69.
46. Nanci A, W.S., Bianco P, *Ten Cate's oral histology: development, structure, and function.* 6th ed. 2003. p.111-43.
47. Rauchenzauner, M., et al., *Sex-and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years.* The Journal of Clinical Endocrinology & Metabolism, 2006. **92**(2): p. 443-449.
48. Parfitt, A.M., *The physiologic and clinical significance of bone histomorphometric data.* Bone histomorphometry: techniques and interpretation, 1983: p. 143-223.
49. Looker, A., et al., *Updated data on proximal femur bone mineral levels of US adults.* Osteoporosis International, 1998. **8**(5): p. 468-490.

50. Martin, R.B., J.C. Pickett, and S. Zinaich, *Studies of skeletal remodeling in aging men.* Clinical orthopaedics and related research, 1980(149): p. 268-282.
51. Grynpas, M., *Age and disease-related changes in the mineral of bone.* Calcified tissue international, 1993. **53**(1): p. S57-S64.
52. Grynpas, M.D., *The concept of bone quality in osteoporosis.* Aging, Osteoporosis, and Dental Implants. Chicago: Quintessence books, 2002: p. 25-34.
53. B., Ö., *Hiperkalsemi Sendromları.* İç Hastalıkları Erol Ç. , 2008. **1.baskı**(Nobel Tıp Kitabevleri): p. 3975-3988.
54. Boyce, B., Z. Yao, and L. Xing, *Osteoclasts have multiple roles in bone in addition to bone resorption.* Critical Reviews™ in Eukaryotic Gene Expression, 2009. **19**(3).
55. Schenk, R., *Bone regeneration: biologic basis.* Guided bone regeneration in implant dentistry, 1994: p. 49-100.
56. Angın, G., *Radyoterapinin gelişimini tamamlamış kemik dokusunda meydana getirdiği hasarların sitoprotektif ajanlar oldukları bilinen amifostin ve karnitin ile önlenmesinin sintigrafik ve histopatolojik parametreler ile değerlendirilmesi.* 2008.
57. Halis Ali ÇOLPAK, N.K., *ALVEOLER KEMİĞİN VERTİKAL YÜKSELTİLMESİ İÇİN KEMİK GREFTİ VE DENTAL PULPA KAYNAKLI KÖK HÜCRE UYGULAMASI İLE EŞ ZAMANLI DENTAL İMPLANT YERLEŞTİRİLMESİ: KOYUN MODELİNDE HİSTOLOJİK VE HİSTOMORFOMETRİK ÇALIŞMA*. 2016, ERCİYES ÜNİVERSİTESİ DİŞ HEKİMLİĞİ FAKÜLTESİ.
58. Goiato, M.C., et al., *Longevity of dental implants in type IV bone: a systematic review.* 2014. **43**(9): p. 1108-1116.
59. Cawood, J., R.J.I.j.o.o. Howell, and m. surgery, *Reconstructive preprosthetic surgery: I. Anatomical considerations.* 1991. **20**(2): p. 75-82.
60. Lekholm, U.J.T.-i.p.O.i.c.d., *Patient selection and preparation.* 1985: p. 199-209.
61. Organization, W.H., *Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: report of a WHO study group [meeting held in Rome from 22 to 25 June 1992].* 1994.
62. Jergas, M. and C.-C. Glüer. *Assessment of fracture risk by bone density measurements*. in *Seminars in nuclear medicine*. 1997. Elsevier.
63. US, D., *Bone health and osteoporosis: a report of the Surgeon General.* http://www. surgeongeneral. gov/library/bonehealth/content. html, 2004.
64. Schmitt, N.M., J. Schmitt, and M. Dören, *The role of physical activity in the prevention of osteoporosis in postmenopausal women—an update.* Maturitas, 2009. **63**(1): p. 34-38.
65. Organization, W.H., *Prevention and management of osteoporosis: report of a WHO scientific group*. 2003: World Health Organization.
66. Tüzün, F., *Osteoporozun Tanımı, Sınıflaması ve Epidemiyolojisi".* Osteoporoz Sempozyumu, 1999.
67. Testi, D.O.R., *Osteoporozun Sınıflandırılması, Risk Faktörlerinin Belirlenmesi ve Bir.* EURASIAN JOURNAL OF FAMILY MEDICINE: p. 107.
68. Meray, J., et al., *Osteoporozda tanı ve tedavi.* Galenos Yayınevi İstanbul, 2012: p. 1-7.
69. Schneider, A. and E. Shane, *Osteoporosis secondary to illnesses and medications*, in *Osteoporosis (Second Edition)*. 2001, Elsevier. p. 303-326.

70. Riggs, B.L., S. Khosla, and L.J. Melton, *The type I/type II model for involutional osteoporosis: update and modification based on new observations*, in *Osteoporosis (Second Edition)*. 2001, Elsevier. p. 49-58.
71. Miller, P.D., S.L. Bonnick, and C.J. Rosen. *Clinical utility of bone mass measurements in adults: consensus of an international panel*. in *Seminars in arthritis and rheumatism*. 1996. Elsevier.
72. Faulkner, K.G.J.R.D.C., *Update on bone density measurement.* 2001. **27**(1): p. 81-99.
73. Akpolat, V.A.J.D.T.D., *Osteoporoz Tanısında Kullanılan Kemik Mineral Yoğunluğu Ölçüm Yöntemleri.* **35**(3): p. 216-220.
74. Garg, A.K., *Bone biology, harvesting, grafting for dental implants: rationale and clinical applications*. 2004: Quintessence Publishing Company.
75. Kelly, T., et al., *DXA body composition: theory and practice.* 1998. **49**(5-6): p. 511-513.
76. Kanis, J.A., *Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report.* Osteoporosis international, 1994. **4**(6): p. 368-381.
77. Sutcliffe, A., *Postmenopausal Osteoporosis: MIMS Pocket Guide.* Haymarket Medical Imprint, London, 2005.
78. MacLaughlin, E.J. and C.L. Raehl, *ASHP therapeutic position statement on the prevention and treatment of osteoporosis in adults.* American Journal of Health-System Pharmacy, 2008. **65**(4): p. 343-357.
79. Pleiner-Duxneuner, J., et al., *Treatment of osteoporosis with parathyroid hormone and teriparatide.* 2009. **84**(3): p. 159-170.
80. Delmas, P.D., *The treatment of postmenopausal osteoporosis*, in *Prevention and Treatment of Osteoporosis in the High-Risk Patient*. 2005, CRC Press. p. 58-82.
81. Watts, N.B., et al., *National Osteoporosis Foundation 2008 Clinician's Guide to Prevention and Treatment of Osteoporosis and the World Health Organization Fracture Risk Assessment Tool (FRAX): what they mean to the bone densitometrist and bone technologist.* 2008. **11**(4): p. 473.
82. Delmas, P.D., *Treatment of postmenopausal osteoporosis.* The Lancet, 2002. **359**(9322): p. 2018-2026.
83. Torgerson, D.J. and S.E.J.J. Bell-Syer, *Hormone replacement therapy and prevention of nonvertebral fractures: a meta-analysis of randomized trials.* 2001. **285**(22): p. 2891-2897.
84. Nelson, H.D., et al., *Postmenopausal hormone replacement therapy: scientific review.* 2002. **288**(7): p. 872-881.
85. Cummings, S.R., et al., *The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial.* 1999. **281**(23): p. 2189-2197.
86. Delmas, P., et al., *Severity of prevalent vertebral fractures and the risk of subsequent vertebral and nonvertebral fractures: results from the MORE trial.* 2003. **33**(4): p. 522-532.
87. Hamdy, R.C., et al., *Review of treatment modalities for postmenopausal osteoporosis.* 2005. **98**(10): p. 1000-1015.
88. A ardan Mutlu N.B., *SERM(Selective estro en receptor modulators) Grubu Raloksifen ve Tamoksifen İçeren Yeni İlaç Şekilleri Üzerine Çalışmalar.* , in *Sağlık Bilimleri Enstitüsü Farmasötik Teknoloji Ana Bilim Dalı*. 2013, Gazi Üniversitesi
89. Bryant, H.U.J.R.i.E. and M. Disorders, *Mechanism of action and preclinical profile of raloxifene, a selective estrogen receptor modulator.* 2001. **2**(1): p. 129-138.

90. Miclea, R.D., et al., *Development and characterization of lipidic cochleate containing recombinant factor VIII.* 2007. **1768**(11): p. 2890-2898.
91. Naot, D. and J.J.B. Cornish, *The role of peptides and receptors of the calcitonin family in the regulation of bone metabolism.* 2008. **43**(5): p. 813-818.
92. Chatziavramidis, A., et al., *Intranasal complications in women with osteoporosis under treatment with nasal calcitonin spray: Case reports and review of the literature.* 2008. **35**(3): p. 417-422.
93. Mayes, S.L.J.N.i.c.p., *Review of postmenopausal osteoporosis pharmacotherapy.* 2007. **22**(3): p. 276-285.
94. Black, D.M., et al., *Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures.* 1996. **348**(9041): p. 1535-1541.
95. Cummings, S.R., et al., *Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: results from the Fracture Intervention Trial.* 1998. **280**(24): p. 2077-2082.
96. Sindel, D.J.T.J.o.P.M. and Rehabilitation, *Osteoporosis treatment at present and in the future/Gunumuzde ve gelecekte osteoporoz tedavisi.* 2013. **59**(4): p. 330-338.
97. Haguenauer, D., et al., *Fluoride for treating postmenopausal osteoporosis.* 2000(4).
98. Adachi, M. and R.J.C.c. Takayanagi, *Effect of anabolic steroids on osteoporosis.* 2008. **18**(10): p. 1451-1459.
99. Kanis, J., et al., *European guidance for the diagnosis and management of osteoporosis in postmenopausal women.* 2008. **19**(4): p. 399-428.
100. O'Donnell, S., et al., *Strontium ranelate for preventing and treating postmenopausal osteoporosis.* 2006(3).
101. Potts, J.T.J.J.o.E., *Parathyroid hormone: past and present.* 2005. **187**(3): p. 311-325.
102. Girotra, M., et al., *The use of parathyroid hormone in the treatment of osteoporosis.* 2006. **7**(1-2): p. 113-121.
103. Stroup, J., M.P. Kane, and A.M.J.A.J.o.H.-S.P. Abu-Baker, *Teriparatide in the treatment of osteoporosis.* 2008. **65**(6): p. 532-539.
104. Locklin, R.M., et al., *Mediators of the biphasic responses of bone to intermittent and continuously administered parathyroid hormone.* 2003. **89**(1): p. 180-190.
105. Rubin, M., et al., *The anabolic effects of parathyroid hormone.* 2002. **13**(4): p. 267-277.
106. Adell, R., et al., *A 15-year study of osseointegrated implants in the treatment of the edentulous jaw.* 1981. **10**(6): p. 387-416.
107. Brånemark, P.-I., G.A.G.A. Zarb, 1938-, and T. Albrektsson, *Tissue-integrated prostheses : osseointegration in clinical dentistry / edited by Per-Ingvar Bra˚nemark, George A. Zarb, Tomas Albrektsson.* 1985.
108. Marco, F., et al., *Peri-implant osteogenesis in health and osteoporosis.* 2005. **36**(7-8): p. 630-644.
109. Linder, L., K. Obrant, and G.J.A.O.S. Boivin, *Osseointegration of metallic implants: II. Transmission electron microscopy in the rabbit.* 1989. **60**(2): p. 135-139.
110. Hayashi, K., et al., *The affinity of bone to hydroxyapatite and alumina in experimentally induced osteoporosis.* 1989. **4**(3): p. 257-262.

111. Qi, M.-C., et al., *Oestrogen replacement therapy promotes bone healing around dental implants in osteoporotic rats.* 2004. **33**(3): p. 279-285.
112. Egermann, M., J. Goldhahn, and E. Schneider, *Animal models for fracture treatment in osteoporosis.* Osteoporosis international, 2005. **16**(2): p. S129-S138.
113. F., S., *Tavşan Femur'unun Morfometrik ve Biyomekanik Özellikleri Üzerine Ovariektominin Etkisi.* Doktora Tezi, Adnan Menderes Üniversitesi Sağlık Bilimleri Entitüsü, 2006.
114. Fini, M., et al., *Osteoporosis and biomaterial osteointegration.* 2004. **58**(9): p. 487-493.
115. Jee, W. and W. Yao, *Overview: animal models of osteopenia and osteoporosis.* J Musculoskelet Neuronal Interact, 2001. **1**(3): p. 193-207.
116. Kimmel, D.B., *Animal models in osteoporosis research*, in *Principles of Bone Biology (Second Edition)*. 2002, Elsevier. p. 1635-1655.
117. Fini, M., et al., *Osteoporosis and biomaterial osteointegration.* Biomedicine & pharmacotherapy, 2004. **58**(9): p. 487-493.
118. Kimmel, D.B., *Animal models in osteoporosis research*, in *Principles of bone biology*. 2002, Elsevier. p. 1635-1655.
119. Y., A., *OSTEOPOROZLU TAVŞAN MODELİNDE SİSTEMİK ZOLEDRONİK ASİT UYGULAMASININ TİTANYUM İMPLANT OSSEOENTEGRASYONU ÜZERİNE ETKİSİ.* Doktora Tezi, Çukurova Üniversitesi Sağlık Bilimleri Enstitüsü 2008.
120. Castaneda, S., et al., *Bone mineral measurements of subchondral and trabecular bone in healthy and osteoporotic rabbits.* 2006. **35**(1): p. 34-41.
121. Alper YILDIZ , E.E., *OSTEOPOROZLU TAVŞAN MODELİNDE SİSTEMİK ZOLEDRONİK ASİT UYGULAMASININ TİTANYUM İMPLANT OSSEOENTEGRASYONU ÜZERİNE ETKİSİ*. 2008, ÇUKUROVA UNIVERSITY.
122. Meredith, N., et al., *Resonance frequency measurements of implant stability in viva. A cross-sectional and longitudinal study of resonance frequency measurements on implants in the edentulous and partially dentate maxilla.* 1997. **8**(3): p. 226-233.
123. Legeros, R.Z., R.G.J.J.o.B. Craig, and M. Research, *Strategies to affect bone remodeling: osteointegration.* 1993. **8**(S2): p. S583-S596.
124. Erinç, Ö. and P.J.O.M.Ü.D.H.F.D. GÜNERİ, *Diş Hekimliğinde Kemik Kalitesinin Belirlenmesi: Radyografik Yöntemler ve Yorumları.* **8**(3): p. 190-199.
125. Obradović, R.R., L.G. Kesić, and S.J.L.i.m.s. Peševska, *Influence of low-level laser therapy on biomaterial osseointegration: a mini-review.* 2009. **24**(3): p. 447-451.
126. Langdahl, B.L. and T.J.T.a.i.m.d. Harsløf, *Medical treatment of osteoporotic vertebral fractures.* 2011. **3**(1): p. 17-29.
127. Hodsman, A.B., et al., *Parathyroid hormone and teriparatide for the treatment of osteoporosis: a review of the evidence and suggested guidelines for its use.* 2005. **26**(5): p. 688-703.
128. Ogita, M., et al., *Differentiation and proliferation of periosteal osteoblast progenitors are differentially regulated by estrogens and intermittent parathyroid hormone administration.* 2008. **149**(11): p. 5713-5723.
129. Lee, J.J., et al., *Successful treatment of advanced bisphosphonate-related osteonecrosis of the mandible with adjunctive teriparatide therapy.* 2011. **33**(9): p. 1366-1371.

130. Luvizuto, E.R., et al., *Osteocalcin immunolabeling during the alveolar healing process in ovariectomized rats treated with estrogen or raloxifene.* 2010. **46**(4): p. 1021-1029.
131. Giro, G., et al., *The effect of oestrogen and alendronate therapies on postmenopausal bone loss around osseointegrated titanium implants.* 2011. **22**(3): p. 259-264.
132. Luvizuto, E.R., et al., *Histomorphometric analysis and immunolocalization of RANKL and OPG during the alveolar healing process in female ovariectomized rats treated with oestrogen or raloxifene.* 2010. **55**(1): p. 52-59.
133. Murthy, P.N., et al., *Effect of ormeloxifene on ovariectomy-induced bone resorption, osteoclast differentiation and apoptosis and TGF beta-3 expression.* 2006. **100**(4-5): p. 117-128.
134. Luvizuto, E.R., et al., *Raloxifene therapy inhibits osteoclastogenesis during the alveolar healing process in rats.* 2011. **56**(10): p. 984-990.
135. Simonet, W., et al., *Osteoprotegerin: a novel secreted protein involved in the regulation of bone density.* 1997. **89**(2): p. 309-319.
136. Lacey, D., et al., *Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation.* 1998. **93**(2): p. 165-176.
137. Kawamoto, S., et al., *Effects of oestrogen deficiency on osteoclastogenesis in the rat periodontium.* 2002. **47**(1): p. 67-73.
138. Jee, W. and W.J.J.M.N.I. Yao, *Overview: animal models of osteopenia and osteoporosis.* 2001. **1**(3): p. 193-207.
139. Khadra, M., et al., *Low-level laser therapy stimulates bone-implant interaction: an experimental study in rabbits.* 2004. **15**(3): p. 325-332.
140. Kalu, D.N.J.B. and mineral, *The ovariectomized rat model of postmenopausal bone loss.* 1991. **15**(3): p. 175-191.
141. Turner, A.S.J.E.C.M., *Animal models of osteoporosis—necessity and limitations.* 2001. **1**(66-81): p. 13.
142. Wronski, T., et al., *Skeletal alterations in ovariectomized rats.* 1985. **37**(3): p. 324-328.
143. Pearce, A., et al., *Animal models for implant biomaterial research in bone: a review.* 2007. **13**(1): p. 1-10.
144. Balatsouka, D., et al., *The impact of nicotine on osseointegration: an experimental study in the femur and tibia of rabbits.* 2005. **16**(4): p. 389-395.
145. O'Sullivan, D., L. Sennerby, and N.J.C.o.i.r. Meredith, *Influence of implant taper on the primary and secondary stability of osseointegrated titanium implants.* 2004. **15**(4): p. 474-480.
146. Cao, T., et al., *Bone mineral density in mandibles of ovariectomized rabbits.* 2001. **12**(6): p. 604-608.
147. Sevil, F., *Tavşan femur'unun morfometrik ve biyomekanik özellikleri üzerine ovariektominin etkisi.* 2006, Adnan Menderes Üniversitesi Sağlık Bilimleri Enstitüsü.
148. Mori, H., et al., *Osseointegration of dental implants in rabbit bone with low mineral density.* 1997. **55**(4): p. 351-361.
149. Lugero, G.G., et al., *Histomorphometric evaluation of titanium implants in osteoporotic rabbits.* 2000. **9**(4): p. 303-309.
150. Doğar, A.G.D.G. and A. Koçak-Büyükdere, *Dental implantlarda stabilite ölçüm yöntemleri The methods of dental implant stability.*

151. Gürsoytrak, B., *İmplant uygulamalarında farklı yüzey özelliklerinin osseointegrasyon üzerine etkisinin rezonans frekans analizi ile değerlendirilmesi*. 2013, Selçuk Üniversitesi Sağlık Bilimleri Enstitüsü.
152. Rozé, J., et al., *Correlating implant stability to bone structure.* 2009. **20**(10): p. 1140-1145.
153. Sjöström, M., et al., *Monitoring of implant stability in grafted bone using resonance frequency analysis: A clinical study from implant placement to 6 months of loading.* 2005. **34**(1): p. 45-51.
154. Oue, H., et al., *Influence of implant surface topography on primary stability in a standardized osteoporosis rabbit model study.* 2015. **6**(1): p. 143-152.
155. GEÇKİLİ, O., et al., *Dental İmplant Yüzeylerinde Osseointegrasyonun Geliştirilmesi için Yapılan Modifikasyonlar Surface Treatments of Dental Implant to Improve Osseointegration.*
156. Peng, Z., et al., *The mechanical strength of bone in different rat models of experimental osteoporosis.* 1994. **15**(5): p. 523-532.
157. Fujimoto, T., et al., *Effects of steroid-induced osteoporosis on osseointegration of titanium implants.* 1998. **13**(2).
158. Jung, Y.-C., et al., *Effects of Ion Beam--Assisted Deposition of Hydroxyapatite on the Osseointegration of Endosseous Implants in Rabbit Tibiae.* 2001. **16**(6).
159. Giro, G., et al., *Effect of 17β-Estradiol and Alendronate on the Removal Torque of Osseointegrated Titanium Implants in Ovariectomized Rats.* 2007. **78**(7): p. 1316-1321.
160. Park, Y.S., et al., *Correlation between microtomography and histomorphometry for assessment of implant osseointegration.* 2005. **16**(2): p. 156-160.
161. Park, S.-Y., et al., *The evaluation of the correlation between histomorphometric analysis and micro-computed tomography analysis in AdBMP-2 induced bone regeneration in rat calvarial defects.* 2011. **41**(5): p. 218-226.
162. Swain, M.V. and J.J.I.j.o.o.s. Xue, *State of the art of micro-CT applications in dental research.* 2009. **1**(4): p. 177.
163. Almagro, M.I., et al., *PTH [1-34] enhances bone response around titanium implants in a rabbit model of osteoporosis.* 2013. **24**(9): p. 1027-1034.
164. Burghardt, A.J., et al., *High-resolution computed tomography for clinical imaging of bone microarchitecture.* 2011. **469**(8): p. 2179-2193.
165. Yakıncı, M.E., et al., *Sağlık, Fen ve Mühendislik Bilimlerinde Mikro-Bilgisayarlı Tomografinin (Mikro-CT) Önemi Importance of Micro-Computed Tomography (Micro-CT) in health, Science and Engineering Sciences.*
166. Freilich, M., et al., *Implant system for guiding a new layer of bone. Computed microtomography and histomorphometric analysis in the rabbit mandible.* 2009. **20**(2): p. 201-207.
167. Monjo, M., et al., *In vivo expression of osteogenic markers and bone mineral density at the surface of fluoride-modified titanium implants.* 2008. **29**(28): p. 3771-3780.
168. Gao, Y., et al., *Effect of combined local treatment with zoledronic acid and basic fibroblast growth factor on implant fixation in ovariectomized rats.* 2009. **44**(2): p. 225-232.
169. Qi, M., et al., *Effect of zoledronate acid treatment on osseointegration and fixation of implants in autologous iliac bone grafts in ovariectomized rabbits.* 2012. **50**(1): p. 119-127.

170. Oki, Y., et al., *Effects of continual intermittent administration of parathyroid hormone on implant stability in the presence of osteoporosis: an in vivo study using resonance frequency analysis in a rabbit model.* 2017. **25**(5): p. 498-505.
171. Corsini, M.S., et al., *Effect of systemic intermittent administration of human parathyroid hormone (rhPTH [1–34]) on the resistance to reverse torque in rabbit tibiae.* 2008. **34**(6): p. 298-302.
172. Heo, H.-A., et al., *Effect of Raloxifene Administration on Bone Response Around Implant in the Maxilla of Osteoporotic Rats.* 2019. **28**(3): p. 272-278.
173. Ramalho-Ferreira, G., et al., *Raloxifene enhances peri-implant bone healing in osteoporotic rats.* 2015. **44**(6): p. 798-805.
174. Kaufman, J.-M., et al., *Teriparatide effects on vertebral fractures and bone mineral density in men with osteoporosis: treatment and discontinuation of therapy.* 2005. **16**(5): p. 510-516.
175. Lindsay, R., et al., *Sustained vertebral fracture risk reduction after withdrawal of teriparatide in postmenopausal women with osteoporosis.* 2004. **164**(18): p. 2024-2030.
176. Rittmaster, R.S., et al., *Enhancement of bone mass in osteoporotic women with parathyroid hormone followed by alendronate.* 2000. **85**(6): p. 2129-2134.
177. Kurland, E.S., et al., *The importance of bisphosphonate therapy in maintaining bone mass in men after therapy with teriparatide [human parathyroid hormone (1–34)].* 2004. **15**(12): p. 992-997.
178. Kimura, S., et al., *Effects of raloxifene and alendronate on non-enzymatic collagen cross-links and bone strength in ovariectomized rabbits in sequential treatments after daily human parathyroid hormone (1-34) administration.* 2017. **28**(3): p. 1109-1119.
179. Cosman, F., et al., *Effects of intravenous zoledronic acid plus subcutaneous teriparatide [rhPTH (1–34)] in postmenopausal osteoporosis.* 2011. **26**(3): p. 503-511.