A CELLULAR COMPARISON OF BONE INDUCTIVE PROPERTIES OF TRABECULAR METAL vs TITANIUM IN HEALTHY AND OSTEOPENIA/OSTEOPOROSIS SUBJECTS

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ABSTRACT

ELIZABETH L. CAMPBELL : A Cellular Comparison of Bone Inductive Properties of Trabecular Metal vs Titanium in Healthy and Osteopenia Subjects.
(Under the direction of Thiago Morelli, Steven Offenbacher and Antonio Moretti)

In this study, we examined the histologic healing associated with initial osseous healing comparing trabecular metal vs. standard titanium in healthy and osteopenia/osteoporosis subjects. We proposed to add to this body of knowledge in two major ways. First, we identified the temporal differences in cellular recruitment and activation during early healing and early osteogenesis, and the bone to implant contact (BIC). This was done by placing test cylinders (approx. 2.9-3 x 5 mm) using standard titanium or trabecular metal. Both total BIC and BIC percentage was calculated for both trabecular and titanium cylinders harvested 4 weeks after placement. Secondly, we recognized that osseous healing can vary considerably in the face of chronic health problems including osteopenia/ osteoporosis. This chronic condition is known to impair bone repair and osseous regeneration. The use of trabecular metal may have the strategic advantage of demonstrating superior osseous healing in these compromised individuals as compared to titanium. This project was designed to examine the differences in the histologic BIC, not only comparing trabecular metal to titanium, but also to examine these differences in subjects with this osseous metabolic compromising condition.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BFM	Brightfield microscopy
BGLAP	Bone Gamma-Carboxyglutamate protein
BIC	Bone to implant contact: %
BMP	Bone morphogenetic protein
BMD	Bone Mineral Density
BOP	Bleeding on probing
BRONJ	Bisphosphonate-related osteonecrosis of jaws
BSAP	Bone-specific alkaline phosphatase
CT	Calcitonin
CTX	C-telopeptide of Type I collagen
DALYs	Disability Adjusted Life Years
DXA	Dual-energy X-ray absorptiometry
EGFR	Epidermal Growth Factor Receptor
ERT	Estrogen replacement therapy
FGFR2	Fibroblast Growth Factor Receptor 2
FN1	Fibronectin 1
FRAX	Fracture Risk Assessment Tool
GEE	General estimating equations
GLM	General linear model
IFO	International Foundation of Osteoporosis

IRB	Institutional review board
MI	Myocardial infarction
MRONJ	Medication-related osteonecrosis of the jaws
MSC	Mesenchymal stem cells
M-CSF	Macrophage colony-stimulating factor
NIH	National Institutes of Health
NTX	N-terminal telopeptide
OC	Osteocalcin
ONJ	Osteonecrosis of jaws
OPG	Osteoprotegerin
PCR	Polymerase chain reaction
PINP	Procollagen type I N-terminal propeptide
PHEX	Phosphate Regulating Endopeptidase Homolog X-linked
PPi	Inorganic Pyrophosphate
PTH	Parathyroid hormone
PTTM	Porous Tantalum Trabecular Metal
RANK	Receptor activator of nuclear factor kappa B
RANKL	Receptor activator of nuclear factor kappa B ligand
RNA	Ribonucleic acid
SEM	Scanning electron microscopy
SERM	Selective Estrogen Receptor Modulator
SERPINH1	Serpin Family H Member 1
SMAD	Small Mothers Against Decapentaplegic gene

Ta	Tantalum
Tb	Trabecular
TGF/BMP	Transforming growth factor/Bone morphogenetic proteins
TGFB	Transforming Growth Factor Beta
TNF-α	Tumor necrosis factor alpha
TPTD	Teriparatide
U.S.	United States
VCAM1	Vascular Cell Adhesion molecule
VDR	Vitamin D (1,25- Dihydroxy Vitamin D3) Receptor
VEGFB	Vascular Endothelial Growth Factor B
VTE	Venous thromboembolic events
WHO	World Health Organization
1,25 Vit D3	Vitamin D3

CHAPTER 1: OSTEOPOROSIS/OSTEOPENIA, BONE AND TISSUE ENGINEERING AND THE ROLE OF DENTAL IMPLANTS IN AFFECTED PATIENTS

Introduction

Osteoporosis is a debilitating metabolic bone disease that is a major health condition that affects approximately 75 million people in the US, Europe and Japan(1). Ten million are affected in the U.S. alone, and by the year 2025 it is predicted that a 50% increase in prevalence will be seen due to the contribution of the aging population(2). It is defined by the World Health Organization (WHO) as a bone density 2.5 SDs below the mean for young adult women(3). Osteoporosis leads to an increased incidence of fracture, most notably hip fractures; leading to an increased mortality and morbidity(3). In patients with hip fractures, 1 in 5 die, while of the total number with osteoporotic related hip fractures, 1/3 require nursing home placement because they are functionally dependent(3). These fractures trigger increased healthy care costs, estimated at $10-15 billion annually(3).

While osteoporosis is not directly a cause of tooth loss, most patients affected with osteoporosis are elderly and may have an increased risk of tooth loss due to prolonged exposure to various factors that are associated with tooth loss. The need for implant therapy can increase as patient ages due to increased risk for tooth loss. These patients can benefit immensely from rehabilitation using dental implants.

The use of dental implants in patients with osteoporosis is a debatable issue, caused by a lack of mineral density, which can affect initial stability. Dental implant placement can also be affected by the increased bone metabolism in favor of resorption, leading to an

altered ability for bone formation. Medications used to treat osteoporosis have been shown to cause a decrease in risk of fracture, however, many lead to oral side effects such as bisphosphonate related osteonecrosis of the jaw (BRONJ) or more recently known as medication related osteonecrosis of the jaw (MRONJ). There are an abundance of clinical studies reporting on the outcomes of implant therapy in patients with osteoporosis, but several fail to differentiate between the effects of osteoporosis and its therapy. Many studies have demonstrated successful osseointegration of dental implants in osteoporotic patients with no contraindication for implant therapy(4-6). However, there are other studies which show that osseointegration of implants in patients with osteoporosis could be negatively affected(7, 8).

Section 1.1 Bone Tissue: Gross Structure, Formation

Bone is a mineralized and dynamic type of connective tissue that has many functions, including: movement, protection, support, hematopoiesis and mineral reservoir(9). This histologically unique tissue is comprised of a complex structure of both organic content and inorganic components(9). The organic components are comprised mainly of type I collagen and various other non-collagenous matrix proteins(9). Despite their location in the body, all bones have similar basic structure. The variability in the overall size and shape of individual bones begins during embryonic development and continues up to the pre-adult stage in growth(9).

The overall organizational structure of bone includes internal and external components that all interact with one another to form a delicate equilibrium. The external structure of bone includes an outer layer of dense fibrous connective tissue membrane known as the periosteum(9). This membrane is comprised of two layers, an inner and outer layer, both of

which are involved in the functionality of this tissue. The outer layer is known as the fibrous periosteum and it is not in contact with the bone and acts as the location for muscle and tendon attachment, along with aiding in nutritional support(9). Cells within the fibrous layer are very densely packed together; lymphatics, vasculature neural tissue are also located in the fibrous layer(9). The inner layer, known as the osteogenic periosteum, is the layer adjacent to the cortical bone surface(9). The inner periosteum functions to maintain the osteogenic potential of the bone during injury or growth. The internal structural elements include a dense outer layer of compact bone which encapsulates the inner supporting network of trabecular bone(9). The porous structure of the trabeculated bone is filled with marrow, which is the vital tissue involved in hematopoiesis(9). A poorly demarcated layer of loose connective tissue, known as the endosteum, lines the internal aspect of each bone(9). The role of the endosteum is a physical barrier between the two bone layers; it also acts as a source of osteogenic cells(9). The combination of all of these aspects makes up the organizational components of bone.

Different cells that comprise the bone have different functions and in health work in congruence with one another to maintain form and function, however these same cells can function in disease when the balance is tipped. Histologically, both compact and trabecular bones are identical and consist of microscopically unique lamellar types, each of which are found in distinct locations with the bone structure. The osteon is the basic organizational unit that consists of concentric lamellae, which form a cylinder of bone surrounding a central vascular channel(9). Osteoblasts are of mesenchymal origin and are mononucleated cells that produce the organic matrix that comprises the bone(9). Osteoblasts not only form the structural matrix proteins, but also secrete a multitude of cytokines and growth factors, which

regulate cellular function and bone metabolism(9). Osteocytes are osteoblasts cells that have become entombed in the mineral matrix they secrete while forming the structure. These cells are located in lacunae and communicate with one another and other cellular types via enclosed channels. These channels are known as canaliculi and allow for the osteocytes to sense the biochemical and mechanical environmental changes within the bone and transmit signals to induce a response(9). Osteoclasts are larger multinucleated bone cells responsible for the bone degradation, a critical function in maintenance, repair and remodeling processes(9). Osteoclasts originate from monocytes/macrophage lineages and differentiate into functional cells when exposed to receptor activator of nuclear factor kappa-B ligand (RANKL), macrophage colony-stimulating factor (M-CSF), and tumor necrosis factor alpha (TNF-α)(10).

Section 1.2: Bone Metabolism

Because bone is a metabolically active tissue, it is in a constant state of flux in response to specific functional and nutritional demands(9). During childhood, bone turnover is much greater than adults, as one ages the rate of turnover rate decreases, yet remains balanced during a state of health. Bone turnover rates for trabecular bone are 15% per year, while cortical bone is approximately 5% per year(9). The cyclical nature of remodeling consists of three consecutive phases: resorption, reversal and formation(11). The resorptive phase is initiated with the migration of partially differentiated mononuclear pre-osteoclast and lasts approximately 2 weeks(11). The main player in the resorptive phase is the osteoclast, which dissolves the bone mineral, and enzymatically degrades extracellular matrix (ECM) proteins(10). The initiation of the resorptive phase is systemically controlled via four main hormones: vitamin D3 (1,25 Vit D3), parathyroid hormone (PTH), calcinontin, and

estrogen(12). Calcitonin, PTH and 1,25 Vit D3 are all secreted with the intention to control the levels of serum calcium to maintain a precise physiological range(12). With the completion of the resorptive phase, mononuclear cells will appear on the surface of the bone and prepare the surface for bone formation by osteoblasts(11). These reversal mononuclear cells provide signals for the differentiation and migration of osteoblasts and can last up to 4 to 5 weeks(11). Specific factors are created by the differentiated osteoclasts and mononuclear reversal cells, or released from the demineralized matrix trigger the initiation of the formation phase(9). The formative phase main cellular constituent is the osteoblast, which produces the organic bone matrix and aids in mineralization(13). As osteoblasts form bone and mature, they produce more OPG and less RANKL, resulting in the inhibitory action of osteoclasts(9). The formation phase can continue for up to 4 months(11). Mechanical force also plays a role in bone remodeling and architecture through local and systemic responses(14). The osteocyte, which a post-mitotic osteoblasts-derived cell, acts as a mechanosensor and an endocrine cell, facilitating the signal transduction in reaction to mechanical or metabolic stimuli(15).

Section 1.3: Bone Metabolic biological pathways and gene expression

The interplay of osteoblastic and osteoclastic function forms a delicate balance created by a multitude of unique and different cytokines, growth factors, and hormones(9). In disease states, however, the balance is tipped leading to pathologic bone formation or loss. Although the transcriptional regulation and signaling pathways are not well understood, the formation bone requires highly controlled biologic pathways stemming from the up-regulation or down-regulation of key genes involved in the differentiation and maturation of osteoblastic cells(16). Different transcription factors are involved in the differentiation

process from mesenchymal stem cells or hematopoietic precursor cell into their respective bone cells(9). Only select transcription factors will be discussed in this review. Osteoblastic phenotyptic commitment is regulated by principal transcription factors, one of which is transforming growth factor/ bone morphogenetic protein (TGF/BMP) and fibroblast growth factors (FGF)(17). The transcription factors RunX2 and Osterix are essential for control of commitment and differentiation of mesenchymal stem cells (MSCs) into osteoblasts(9). The threshold of RunX2/Osterix will determine the pathway of commitment to either osteocyte or chondrocyte cell lineage(17). Several hormones and cytokines are involved in a dosage and time dependent fashion, influencing the progression of MSCs to an osteoblastic lineage either directly or indirectly(18). Receptor-activated nuclear factor (RANK) and its ligand (RANKL), expressed on the plasma membrane of osteoblast progenitor cells(9). RANKL then binds to the RANK expressed on plasma membrane of osteoclast progenitor cells, inducing the differentiation of osteoclasts(9). The osteoclasts also secrete a decoy to RANKL, known as osteoprotegrin (OPG), which blocks the binding of RANK and RANKL, creating a type of self-regulatory function(9). Localized control of the remodeling ensures that bone is removed when damaged and replaced where needed(19). Fibroblast growth factors (FGF) are growth factors that are involved in the regulation of cellular proliferation, differentiation, survival, and migration of osteoblasts(20). An osteocytic marker, phosphate-regulating gene with homolog X chromosome (PHEX), is one of the many "mineralization-related genes" which are involved in the regulation of mineralization and phosphate metabolism(21). The genes discussed in this section and their ontology classification is shown in Table 1.1.

Gene Name	Gene Ontology Classification
ALPL	Skeletal system development, biomineral tissue development
BGLAP	Regulates bone remodeling and energy metabolism
BMP4	Osteoblast differentiation, positive regulation of pathway-restricted SMAD protein phosphorylation, positive regulation of bone mineralization
TGFB3	SMAD protein import into nucleus, cell proliferation
FGFR2	Influences mitogenesis and differentiation
VCAM1	Mediates leukocyte-endothelial adhesion and signal transduction
EGFR	Binds to epidermal growth factors, induces cell proliferation
VDR	Involved in mineral metabolism
PHEX	Involved in bone mineralization and renal phosphate reabsorption
VEGF-B	Regulates the formation of blood vessels
ITGA1 & ITGA2	Involved in cell-cell adhesion
COL	Skeletal development
CTSK	Proteolysis, bone resorption
SERPINH	Plays a role in collagen biosynthesis, endochondral ossification
FN1	Extracellular matrix structural constituent, angiogenesis, cell adhesions, cell migration, regulation in cell shape

Table 1.1: Molecular assessment of osseointegration. Adapted from Thalji & Cooper 2013(22).

While the mechanisms involved in the local control and systemic control of bone remodeling is not completely understood, the current known major mechanisms have been reviewed in the most basic sense.

Section 1.4 Osteoporosis: Definition, types and pathophysiology

The National Institute of Health (NIH) defined osteoporosis as a "skeletal disorder characterized by compromised bone strength predisposing a person to an increased fracture risk"(3). It has been described by some as a multifactorial age related bone disease that is

characterized by low mineral density, microarchitecture deterioration along with changes in the mineral property of the bone causing enhanced fragility, ultimately leading to a higher susceptibility for fracture(23). Bone strength, density and quality are all utilized as measurements to evaluate the effect of bone metabolic diseases, specifically osteoporosis; all are interdependent on one another. Bone strength is the echoes of the integration of bone density and quality, while bone density is simply expressed in units: grams per area or volume(3). Bone quality is the architecture, mineralization, damage accumulation, and turnover (3). There are two types of osteoporosis, primary and secondary. Primary osteoporosis most often occurs after the onset of menopause, however, it can occur in both sexes and generally is seen later in life for males(3). Secondary osteoporosis is due to the effects of specific medications such as glucocorticoids, diseases or disorders causing malabsorption(3). Primary osteoporosis mainly affects the trabecular bone, while secondary osteoporosis is characterized by the loss of cortical and trabecular bone(24). The micro-architectural differences can be seen upon gross histologic evaluation and with more detail utilizing scanning electron microscopy (SEM).

The pathophysiology of osteoporosis is multifactorial, but stems from the inability of the formative bone phase to outpace the resorptive bone phase, leading to an overall loss of mineral and detrimental changes in microstructure. In health, targeted remodeling is completed in order to repair sites of micro-damage, while stochastic remodeling is completed to maintain plasma homeostasis(25). If stochastic remodeling is excessive it can lead to skeletal weakness through loss of bone mass(25). While remodeling favors the resorptive phase, trabeculae become thinner and fewer in number as well as lose their connectivity(26). Skeletal mass and density will then have been affected to the point of structural instability,

leading to increased risk for fracture(27). An assessment of the remodeling rates can be done with the use of markers of bone resorption: N-terminal telopeptide (NTX) or C-telopeptides of type I collagen (CTX), and markers of bone formation: OC, procollagen type I N-terminal propeptide (PINP) and bone-specific alkaline phosphatase (BSAP); can be used to evaluate increased fracture risk, however, these are difficult apply clinically in patients(25).

Section 1.5: Diagnosis, Prevalence and Burden

The changes in bone mineral density (BMD) microarchitecture that lead to fractures drastically impact the quality of life and can lead to temporary or permanent disability(28). Therefore, the diagnosis of osteoporosis is extremely important in the clinical setting to prevent fractures and decrease fracture risk. In a clinical setting, the utilization of patient history and physical exam coupled with diagnostic tests are key to an accurate diagnosis and evaluation for intervention. In many cases, the disease can be undiagnosed until the patient has suffered from a fracture. The diagnosis of osteoporosis is given when the BMD exceeds more than 2.5 standard deviations (SD) below the normal mean for a young adult woman(3). BMD is reported using T-scores, and a routine and noninvasive method for the measurement of BMD is done by dual energy X-ray absorptiometry (DXA), it is also considered the gold standard for BMD assessment(29). A BMD T-score between 1.0 and 2.5 SDs below the young adult woman mean equates to a diagnosis of osteopenia, while a BMD T-score equal to or above -1.0 is reflects as a normal score(30). A test for BMD will also produce a Z-score that allows for the comparison of the patient's BMD to that of a healthy age matched individual. Both gender and ethnicity/race play a role in the prevalence and fracture incidence(3). The ranges for both T-scores and Z-scores can be seen in Table 1.2.

BMD Scoring	Category
Z-score ≤ 2.0	Below expected range for age and sex match
Z-score > 2.0	Within expected range for age and sex match
T-score ≤ 2.5 below normal	Osteoporosis
T-score 1.0 to 2.5 below normal	Osteopenia
T-score ≥ 1.0 below normal	Normal/Healthy

Table 1.2: BMD Diagnostic Categories. Adapted from Leslie et al 2006 (31).

All ages, races and ethnicities can be affected by osteoporosis, however, the majority are post-menopausal white women(3). Although both men and women show an age-related decline in BMD towards the beginning of midlife, women have a much more pronounced BMD loss after the onset of menopause, which occurs roughly around the age of 51 for most women(3). Menopause is defined as the reduced secretion of estrogen and progesterone, leading to the cessation of menstruation(32). It is diagnosed 12 months after amenorrhea not resulting from a pathological cause, but can be the result of surgical intervention, chemotherapy or radiation(32). With the depletion of estrogen, the inhibition of osteoclasts is lost, along with decreased intestinal calcium absorption caused by the flux of calcium into the plasma from bone resorption resulting in the reduction in parathyroid hormone levels(33). The damaging effects of osteoporosis on BMD can be seen 5 to 7 years surrounding the onset of menopause, whereby women lose approximately 12% of their total bone which is the equivalent to 1 T-score measured by DXA. To help combat modifying factors and increase

the effectiveness of the fracture risk assessment, WHO developed the Fracture Risk Assessment Tool (FRAX)(34). FRAX utilizes risk factors that were identified from several meta-analyses; its algorithms estimate a 10-year probability of fracture(34). Risk factors for fracture include age (40-90 years), weight, height, sex, low femoral neck BMD, history of previous fracture, parental history of hip fracture, current tobacco habits, use of glucocorticoids, rheumatoid arthritis, alcohol intake and other causes attributed to secondary osteoporosis(35). FRAX will then provide a numerical value which is the ten year probability of a major osteoporotic fracture as a percentage and the decision for intervention can be determined from the management chart seen in Figure 1.1(35). With osteoporosis related fractures comes numerous medical related expenses, estimated at 15 to 20 billion dollars yearly in the US which, much of the cost is paid by Medicaid and Medicare(36). Osteoporosis related fractures account for more combined deaths and morbidity than any single type of cancer, with the exception of lung cancer(37). The global burden of osteoporosis is normally quantified by disability adjusted life years (DALYs), which factors the years of life lost due to fracture and the disability of those who survive(38). It was calculated that in 2000, an estimated 9 million osteoporosis related fractures accounted for a total of 5.8 million DALYs(37). This number encompasses 0.83% of the world-wide burden for non-communicable disease(37).

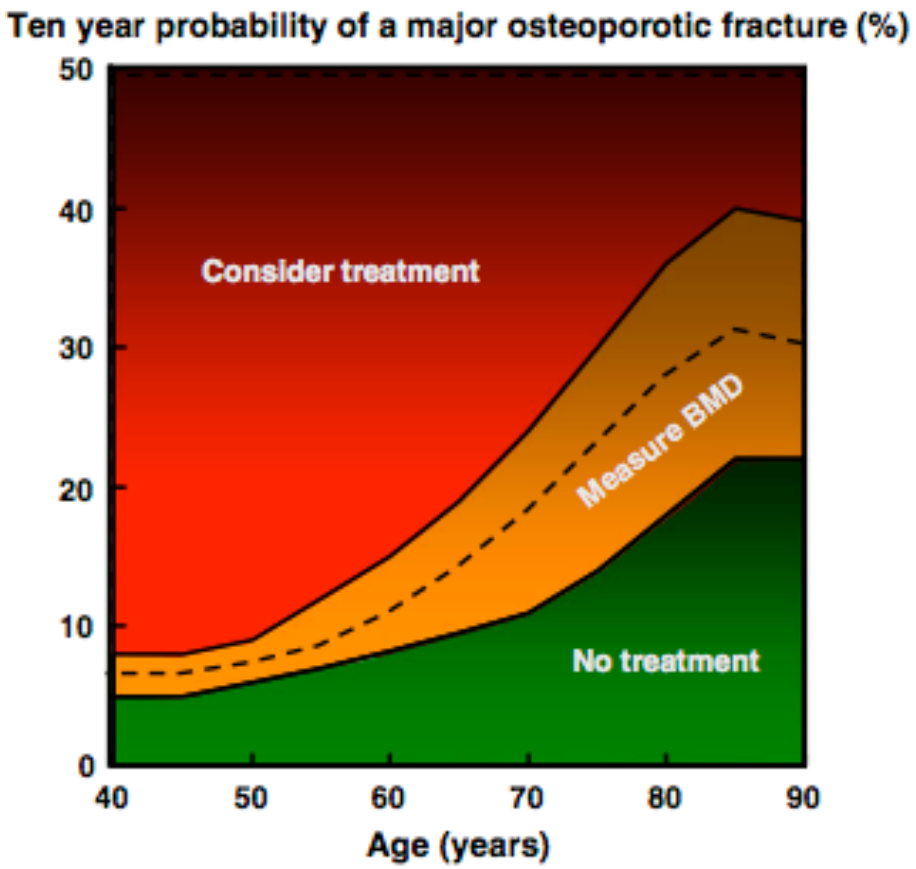

Figure 1.1: Management Chart for Osteoporosis. Dotted line shows the intervention threshold. Reproduced with permission.(39).

Section 1.6: Treatment of Osteoporosis

Osteoporosis is treated in a varying number of ways, including changes in lifestyle approaches and pharmacological interventions. Lifestyle changes range from smoking cessation, weight bearing exercises, calcium and vitamin D supplementation. However, if the patient falls into an increased risk category based on their T/Z-scores or other risk assessment tool, pharmacologic intervention is necessary. Prior to pharmacologic intervention, the cost, risk of adverse effects versus benefit, and limitation of the medication should all be considered(40). The main objective of each pharmacologic treatment modality is to increase bone mass and prevent further loss. The following medication categories have

been utilized in the treatment of osteoporosis: antiresorptive agents and bone-forming agents. Antiresorptive agents include Hormone Replacement Therapy (HRT), bisphosphonates, calcitonin, parathyroid hormone (PTH), RANKL inhibitor, and selective estrogen receptor modulators (SERMs)(41). The current bone-forming agent available on the market is teriparatide. Both antiresorptive and bone-forming agents have a range of dosing frequencies and routes of administration that are tailored specifically for each medication(41). With the use of pharmacologic intervention, vertebral fracture risk is reduced by 30-70%, dependent on the agent and level of adherence(41). The decision to prescribe a pharmacologic intervention or utilize life style changes or dietary supplementation needs to be completed after careful evaluation of the individuals' current BMD score and relative risk.

Bisphosphonates historically have been one of the most frequently used osteoporotic treatment medications. The chemical structure of bisphosphonate is similar to inorganic pyrophosphate (PPi), allowing this type of drug to perform partially as a nonhydrolyzable PPi analog(42). The chemical structure of several bisphosphonates and PPi can be seen in Figure 1.2 below.

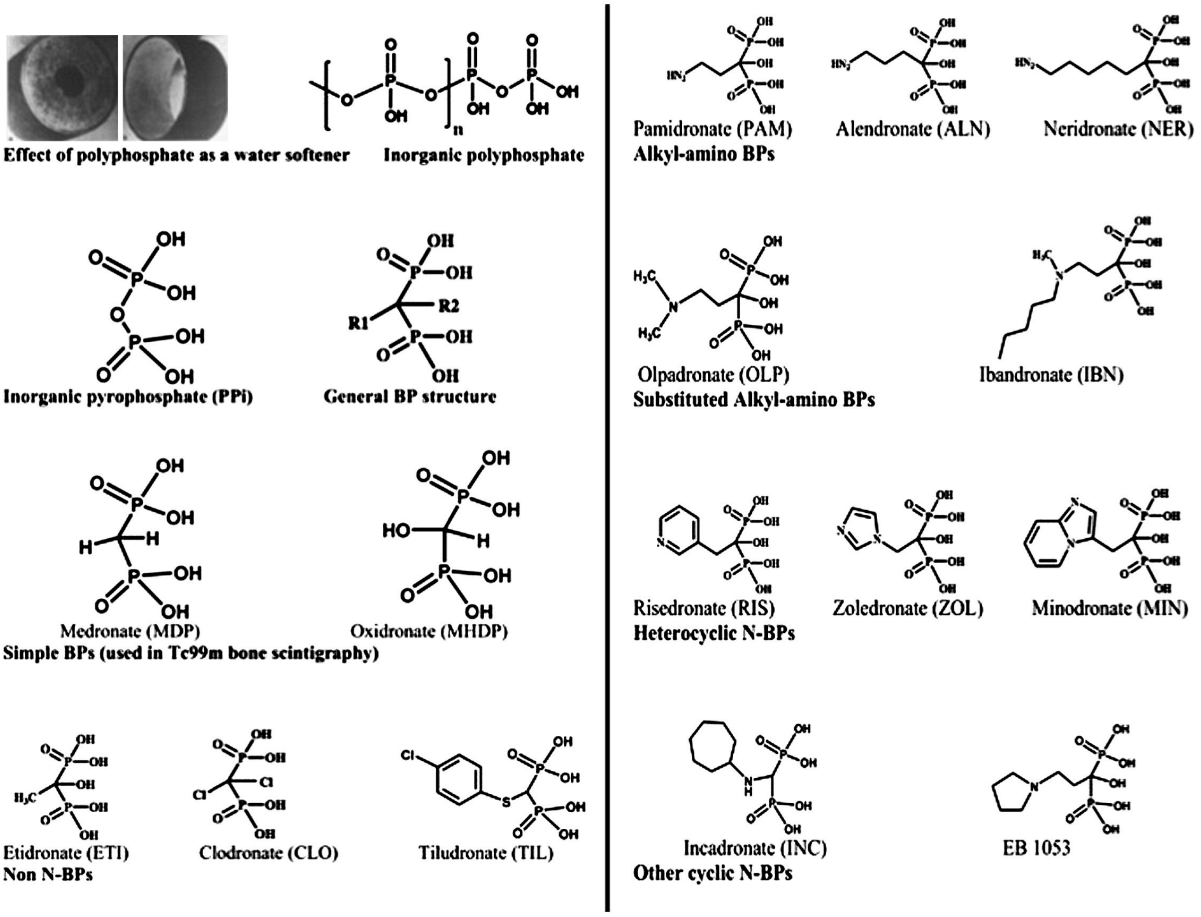

Figure 1.2: Chemical Structure of Bisphosphonates and Inorganic Pyrophosphate. Reproduced with permission. (43).

In general, bisphosphonates are very hydrophilic with poor lipophilicity relating to poor oral bioavailability with only <1% is absorbed from the gastrointestinal tract after oral administration(44). To offset the poor absorption, increased doses can be given as bisphosphonates have a dose-dependent absorption(44). The overall mechanism of bisphosphonates includes the localization of the drug to the bone where they bind to the calcium in hydroxyapatite due to their high affinity for bone, as they are synthetic analogues to PPi(45). The chemical structure of bisphosphonates greatly affects their function; the hydroxyl groups are critical for the binding to calcium and the terminal functional group bound to the central carbon determines the resorption inhibition potency(45). It has also

been shown that bisphosphonates can prevent apoptosis of osteocytes and osteoblasts via the rapid activation through phosphorylation of extracellular signal regulated kinase (ERK) pathway(46). As remodeling occurs, the drug is incorporated into osteoclast, causing reduced resorptive activity and eventually cell death(47). The ability to be preferentially incorporated into the bone and obtain skeletal retention is based on the availability of hydroxyapatite binding sites(45). The hydroxyapatite binding sites are more readily available in bone metabolic disorders that favor resorption(45). Any drug that is not incorporated into the skeleton is then rapidly cleared from circulation by renal excretion(45).

There are two types of bisphosphonate types, nitrogenous and non-nitrogenous. The first generation bisphosphonates are non-nitrougenous and include: etidronate, clodronate and tiludronate(45). These non-nitrogen containing bisphosphonates become incorporated into newly formed adenosine triphosphate (ATP) molecules which accumulate inside osteoclasts and are cytotoxic due to their inability to by hydrolyzed, leading ultimately to cellular apoptosis(45). Second and third generation are nitrogen-containing and include: alendronate, risedronate, ibandronate, pamidronate and zoledronic acid(45). These later generation bisphosphonates bind and inhibit a key regulatory enzyme critical to the production of cholesterols, isoprenoid lipids and other sterols(45). This inhibition leads to interference in key cellular function in osteoclasts, leading to apoptosis(45). The mechanism of the different types of bisphosphonates can be seen in Figure 1.3, below.

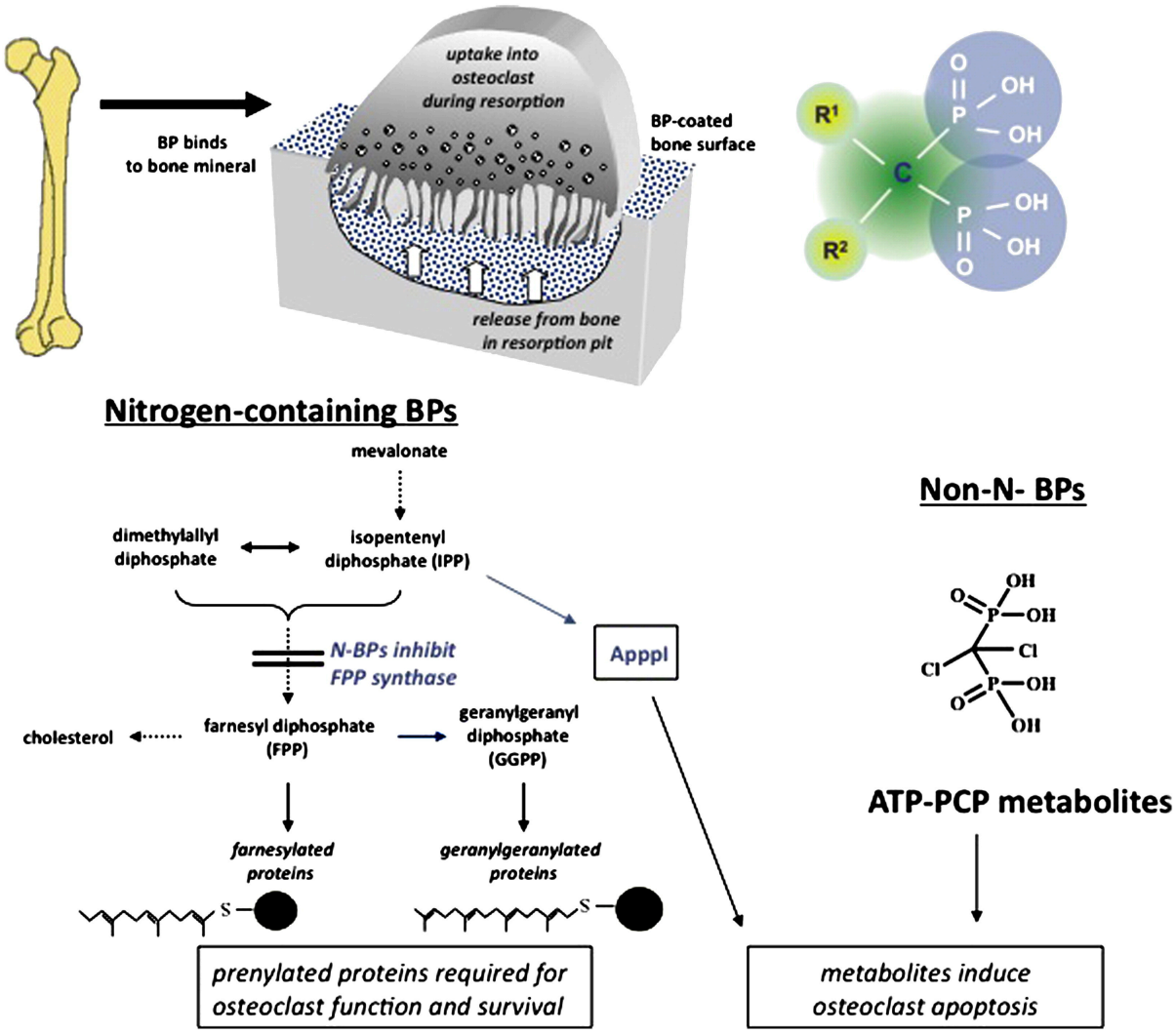

Figure 1.3: The cellular and biochemical mechanisms of action of bisphosphonates. Reproduced with permission. (43).

The route of administration of bisphosphonates is important for dosage, onset and risk for potential side effects. The maximum effects of resorption suppression can be seen around 3 months after the initiation of the oral bisphosphonate(45). The rate of bone remodeling dictates the half-life and due to the length of natural bone turnover the half-life can be at least 10 years(44). These long half-lives are the reason that a drug holiday would not allow for the recovery of osteoclastic function and return of normal bone turnover(45). The effect of binding affinity of different bisphosphonates and the relative uptake and detachment from the osseous surface can be visualized in Figure 1.4.

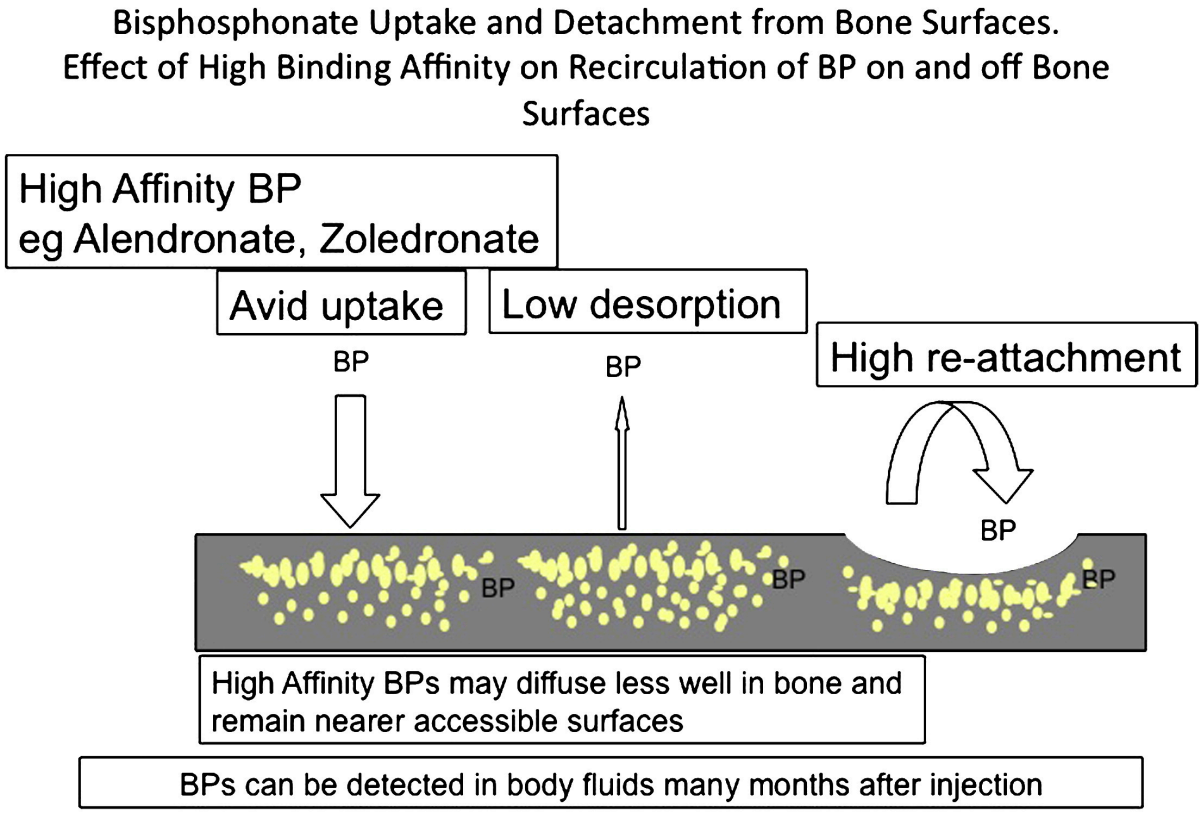

Figure 1.4: Effect of Binding Affinity of Bisphosphonates on their Uptake and Detachment from bone surfaces and their re-cycling. Reproduced with permission. (43).

Bisphosphonates have been associated with various adverse side effects and safety concerns. Some of the adverse side effects are based on the mode of administration; oral administration has been associated with gastrointestinal effects(45). However, acute phase reactions including fever, myalgia, arthralgia and headache are more common with intravenous (IV) administration of bisphosphonates(45). One of the most significant adverse events with severe oral implications is osteonecrosis of the jaw (ONJ), which is thought to be caused by an over suppression of bone turnover(48). This over-suppression allows for the persistence of micro-damage and micro-fractures that accumulate over time(49). Even with the limited osteoclastic activity, osteoblastic activity is still affected, resulting in bone formation that is

rigid and brittle, with an osteoporotic architecture(50). The majority (94%) of reported cases have been in patients with intra-venous (IV) second and third generation bisphosphonate therapy for the treatment for metastatic bone diseases(48). Of the areas in the mouth that are affected, there is a higher propensity for the mandible to be affected (2:1 ratio) as compared to the maxilla(48). More than half of the patients affected (60%) reported a dento-alveolar surgical procedure prior to onset, however, spontaneous onset without injury or surgical intervention can occur(51). The fragility of the mucosal barrier against trauma and increase exposure to oral pathogens are reasons for increase susceptibility for osteonecrosis of the oral cavity(45). The most important risk factors for BRONJ are the total dose, history of trauma, dental surgery or dental infection.

The most abundant mineral in bone is calcium; bone acts as a reservoir for the storage of over 98% of the body's total calcium(52). The majority of peak bone mass is genetically determined and acts as a significant risk determinant for future fracture risk(52). The main advantageous effects of calcium supplementation in the increase of BMD and reduction in fracture risk is most pronounced in the later postmenopausal years, while there is less of an effect in the early post menopausal years(52). Research has shown that calcium supplements alone improve BMD but are unsuccessful in their ability to reduce the risk for fracture(53). Unfortunately, the use of calcium supplementation is not without risk as evidence shows calcium supplementation has an increased risk of cardiovascular events such as myocardial infarction (MI)(53).

Many practitioners have extensively used vitamin D and calcium supplementation as an intervention for at-risk patients; however, many overlook this need prior to or during bisphosphonate therapy. Hypovitaminosis D is more common in patients taking

bisphosphonates, due to limited dietary absorption and reduced intake, possible renal impairment and higher incidence for limited sun exposure(45). Although currently there is no consensus on the optimal 1,25 Vit D3, however 30ng/mL, and reduced vitamin D serum levels cause a reduction in calcium absorption (10-15%) and phosphorus absorption (60%) (54). Hypovitaminosis D can also lead to skeletal muscle weakness which can increase the risk for falls, increasing the risk for fractures(54). With the drop in intestinal calcium absorption there is an increase production in parathyroid hormone increasing the skeletal leaching of calcium to supplement low serum levels(54). The metabolism of vitamin D in the regulation of calcium, phosphorous and bone metabolism can be seen in Figure 1.8.

An endogenous polypeptide hormone used in the treatment of postmenopausal osteoporosis through its affect on osteoclasts is calcitonin (CT)(55). With specific receptors on osteoclasts, the binding of calcitonin causes the movement out of the resorptive pit with the loss of the brush border; it also modifies the internal structure of osteoclasts by inhibiting multiple cytoplasmic functions key to bone resorption(56). Calcitonin shortens the lifespan of number of osteoclasts, it also blocks the union of mononuclear marrow progenitor cells that become osteoclasts, thereby reducing the number of osteoclasts(57). More recently research has shown that calcitonin aid in increasing the survival rate of osteoblasts and osteocytes(46). Early calcitonin was administered via injection, leading to issues in compliance and the need for the creation of suppository and nasal spray modes of administration(58). The effectiveness of calcitonin on osteoporosis treatment has shown a 30% reduction in hip fractures(59). It has also been suggested that calcitonin may have an analgesic effect on patients with acute vertebral fractures, removed from its effect on osteoclasts(60). It has been suggested an osteoclastic resistance to prolong administered

calcitonin can occur and that intermittent administration may help to avoid tolerance(61). Unlike bisphosphonates, the effects of calcitonin on osteoclasts is reversible and the medication does not persist in the bone even after longlapses in medication administration(62).

Teraparatide (TPTD) or recombinant parathyroid hormone is an anabolic therapy was the first FDA approved medication for the treatment of osteoporosis that stimulates bone formation rather than BMD reduction(63). TPTD is recommended for the treatment of osteoporotic female patients with a high fracture risk and male patients at high fracture risk due to primary or hypogonadal osteoporosis. Parathyroid hormone in the body controls calcium serum levels through bone resorption or bone formation, with a sustained exposure results in net bone loss, while bone gain is due to intermittent exposure(63). Teraparatide (TPTD) is a modified parathyroid hormone that works by directly stimulating bone formation and increases the initiation of novel remodeling sites(64). TPTD has several additional effects on bone. TPTD stimulates the proliferation of cells in the osteoblastic lineage through the activation of calcium protein kinase(65). Through increased expression in the Wnt signaling, there is an increase in bone formation and reduces apoptosis of osteoblasts(66). It has been shown to can increase the amount of tracbecular bone and cortical thickness with superior trabecular microarchitecture. Due to some of the concerns with the formation of osteosarcoma formation, TPTD is recommended for a maximum duration of 24 months to limit the risk(64). Additionally, nausea, leg cramps and dizziness are the most commonly reported side effects with TPTD administration. TPTD has also been linked to life threatening hypercalcemia which leads to a multitude of symptoms including arrhythmias, coma and renal dysfunction(67). The influence of PTH on bone metabolism

can be seen in figure 1.5 below. f

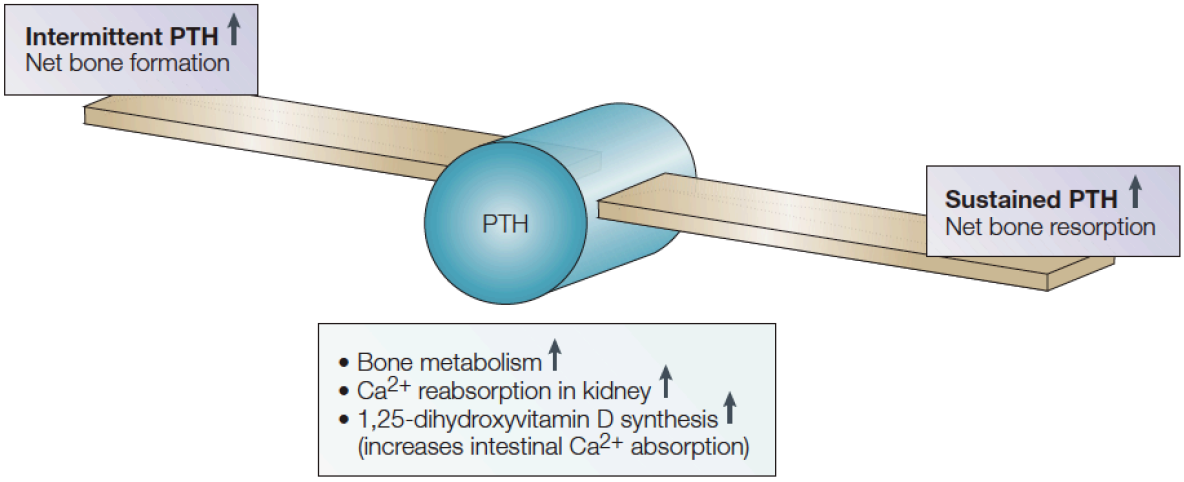

Figure 1.5: Effects of parathyroid hormone. Reproduced with permission(63).

Denosumab is a unique pharmacological intervention that is a fully human immunoglobulin (Ig) G2 monoclonal antibody for RANKL, leading to the inhibition of osteoclastic activity, and increases BMD(68). Denosumab has a high affinity and specificity to RANKL, mimicking endogenous osteoprotegrin (OPG)(69). It is administered via subcutaneous injection once every 6 months(70). The mechanism of Denosumab is that it binds RANKL, blocking its activity with osteoclastic RANK receptors(68). The interaction of RANK with RANKL is key for the development, function and survival of osteoclasts(71). Denosumab has been shown to be as effective as the most efficacious bisphosphonate and has been shown to further reduce the rate of bone metabolism even in patients previously taking bisphosphonates(70). Adverse effects include symptomatic hypocalcemia if not corrected prior to treatment and risk of ONJ, more recently categorized as medication related osteonecrosis of the jaw (MRONJ)(69).

Hormone Replacement Therapy has been utilized in the treatment of osteoporosis due to its ability to significantly reduce fractures, however, several serious adverse reactions have

limited its use(72). Hormone replacement consists of either estrogen as a single medication or as estrogen and progesterone combined. Originally, HRT was prescribed to combat the associated symptoms and was thought to have added cardiovascular benefits(73). With the use of HRT, its affect on BMD and fracture reduction was seen to be valuable in the treatment of post-menopausal osteoporosis. The use of HRT waned after the severe side effects, including increased risk for breast cancer and cardiovascular events(74).

Selective estrogen receptor modulators (SERMs) were developed for the treatment of breast cancer, however today; this class of drug is used for the treatment of breast cancer, osteoporosis and other postmenopausal symptoms. SERMs are tissue selective estrogen agonists for bone, however, it can act as antagonists to estrogen depending on the type(75). In the treatment of osteoporosis, SERMs work on estrogen receptors, down-regulating the activity of osteoclasts and ultimately lessening bone resorption(75). Multiple clinical trials concluded that SERMs aid in the maintenance of BMD, however, the fracture reduction risk is anatomically limited(75). Unlike BPs, SERMs have not been shown to have a continued effect on bones and once SERM administration is suspended the effects on BMD can no longer be seen(75). Side effects include menopausal-like symptoms such as hot flashes as well as venous thromboembolic events (VTEs) and cardiovascular events, as well as an increased risk of endometrial cancer(76).

Several drug types are utilized in the armamentarium for the treatment of osteoporosis, each with a unique mechanism of action. However, the clinical benefit versus the risk must be weighed prior to administration of the drug or combination of drugs. Due to the rapid bone turnover in the jaws, many of these mediations may have more pronounced effects in the oral cavity. Because some drugs can have oral manifestations and may affect the

outcome of implant therapy, precaution should be taken to inform the patient of potential risks. An overview of the mechanism and safety concerns can be seen in Table 1.3 below.

Medication	Mechanism	Safety Concerns
Bisphosphonates	Binds to hydroxyapatite, induces osteoclast apoptosis, can decrease osteoblast apoptosis	BRONJ(48), esophageal cancer (77)
Selective estrogen receptor modulators (SERMs)	Agonist for the estrogen receptor in bone	Venous thromboembolic events(76)
Hormone Replacement Therapy	Prevents osseous changes triggered by estrogen withdrawal	Coronary Heart diseaese(74), Breast cancer, VTE (78)
Calcitonin	Reversibly binds to osteoclasts and inhibits bone resorption	Cancer (79)
Teriparatide	Binds to osteoblasts and stimulates osteoblast activity more than osteoclast activity	Osteosarcoma(64) Leg cramps, hypercalcemia
Denosumab	Prevents interaction of RANK with RANKL. Reduces osteoclast differentiation, survival and activity	MRONJ (69)
Calcium **Vitamin D supplementation**	Provides calcium for bone remodeling, maintains blood calcium Aids in calcium, phosphorous regulation	Kidney stones (80), cardiovascular events (81) Chronic toxicicty at 50,000IU/day(82)

Table 1.3: Osteoporosis therapies, mechanism of action and safety concerns.

Section 1.7: Dental Implants and Osseointegration

Dental implants are a popular replacement in the event of tooth loss or congenitally missing teeth. Placement and restoring dental implants have become more predictable as implant design and surfaces have evolved, however, even with these advances, between 3-10% of implant failures still occur(7, 8). Implants are embedded surgically in the jaw and are fixed initially in bone due to a friction fit known as primary stability, and after remodeling, secondary stability occurs. Osseointegration is defined as the "direct structural and

functional connection between ordered living bone and the surface of the load-covering implant"(83). Later it was described as a healing process dependent on time, whereby a rigid fixation of a bio inert material is clinically asymptomatic and can maintain a functional load(84). Implant fixation is of critical importance to obtaining sufficient osseointegration of a dental implant and help prevent failure in stability(85). When an implant is placed, there is injury to the bone and remodeling must occur in order for the implant to survive. Characteristically around implants, *de novo* bone formation occurs by way of the intramembranous pathways as opposed to an endochondral pathway(86). Osteogenesis around an implant is analogous to osteogenesis during fracture repair; initial formation and stabilization of a clot, followed by inflammatory phase, then proliferative/reparative phase, and finally a remodeling phase(87). In the initial formation and stabilization of a clot, local plasma proteins from the blood are adsorbed on the surface of the implant, setting into motion the clotting cascade(88). The initiation of the clotting cascade allows for the activation of platelets and the release of various cytokines, which are important in angiogenesis, collagen synthesis, and bone turnover(88). The migration and aggregation of neutrophils to the osteotomy herald the start of the inflammatory phase at around 3-4 days post implant surgery(88). Neutrophils are then slowly replaced by macrophages which occurs around 5-6 days post implant surgery(88). The proliferative phase is marked by angiogenesis which allows for the localization of nutrients and cytokines to induce mesenchymal cells to differentiate into fibroblasts and osteoblasts, to form an immature connective tissue matrix(89). Over time, the matrix becomes more mature and more organized. Remodeling is the coupling of the resorption and deposition of bone through osteoclastic and osteoblastic activity respectively(87). It is important to note that

osteogenesis occurs in two locations after dental implant placement; on the surface of the old bone, known as distance osteogeneisis; and on the surface of the implant, known as contact osteogenesis(87).

Initial mechanical stability is another vitally important aspect for proper osseointegration, as micromotions of 150μm or more lead to fibrous encapsulation of the dental implant(90). A fibrous encapsulation prevents intimate contact of bone onto the implant surface, creating a complete lack of osseointegration, leading to failure. The formation of peri-implant bone is normally assessed utilizing multiple parameters including volume, architecture, and bone to implant contact as a fraction of the total implant surface. Originally, the gold standard for the evaluation of osseointegration was microscopic or histologic analysis, however this cannot be completed outside of clinical research(91). Currently, the use of radiographic comparison, cutting torque resistance, reverse torque, modal analysis and resonance frequency analysis have all been proposed as new methodologies for the evaluation of implant osseointegration(91). Any systemic condition, factor or medication that affects any phase of osseointegration can, by default, affect the success of osseointegration and impair implant success and/or survival. On the other hand, implant failure is defined as the first occurrence for which the quantitative performance of a specified implant falls below a specified acceptable limit(92).

Section 1.8: Outcomes and Factors Affecting Dental Implants

Systemic conditions such as diabetes mellitus, osteoporosis and cardiovascular disease can affect implant success and/or survival by increasing the patient's susceptibility to other disease or by causing a malfunction in the healing process(93). Although there are a handful of systemic factors that have been linked to the increased failure of dental implants, there is

no one systemic condition that is an absolute contraindication for dental implant therapy(8). With this being said, the overall success rate of dental implants is quite high, ranging upwards to 98%, while cumulative survival rates were 94.6% after a follow up of over 13 years(94). The definition of success and survival for implants differs slightly and depends specifically on the criteria of the article in question. In general, implant success criteria has not changed much and remains that a single unattached implant remain immobile when tested, lack of radiographic peri-implant radiolucency, vertical bone loss less than 0.2mm after the first year of loading, absence of pain or irreversible signs and symptoms(95). While implant survival is defined as the physical retention of the implant in the patient's mouth, lacking physical removal from the oral cavity(96). Therefore, implant success requires certain criteria be met that involve function, patient satisfaction and physiology(97). While implant survival does not have to meet all the criteria other than function(98).

Section 1.9: Implications of Osteoporosis and Dental Implant Therapy

Patients diagnosed with osteoporosis undergo a variety of skeletal changes which can impact the ability to place implants without prior augmentation procedures due to increased alveolar ridge resorption(99). Reports also indicated that patients with osteoporosis have an altered trabecular pattern in posterior aspects of the mandible and anterior aspects of maxilla(100). It has also been shown that osteoporotic patients demonstrate increased resorption and thinning of the mandibular inferior cortical margin(101). In addition, there have been anecdotal reports indicating an increased incidence of maxillofacial fractures in osteoporotic patients with dental implant therapy(102). Osteoporosis and various other systemic diseases have fallen into the category of relative contraindications, and the clinician must evaluate the patient to determine if they are a candidate for implant therapy based on

stability of the systemic condition and assessing if medications used to treat the condition may interfere with implant outcomes(103). Osteoporosis is notable in that fact that it is subject to controversy for its importance and effects on dental implant outcomes, and has been debated if it affects implants outcomes. The current bone quality classification consists on a scale of 1 to 4 based on the amount of cortical and trabecular bone present. Type 1 bone is a homogenous dense cortical bone throughout the entirety of the implant site, while type 2 bone consists of a thick outer cortical layer surrounding a core of dense trabecular bone(104). Type 3 bone consists of a thin layer of compact cortical bone surrounding a core of dense trabecular bone, while type 4 bone is made of a thin layer of cortical bone surrounding trabecular bone with low density(104). The differences in bone quality types as well as the different jaw shapes related to factors affecting implant placement can be seen in figure 1.6.

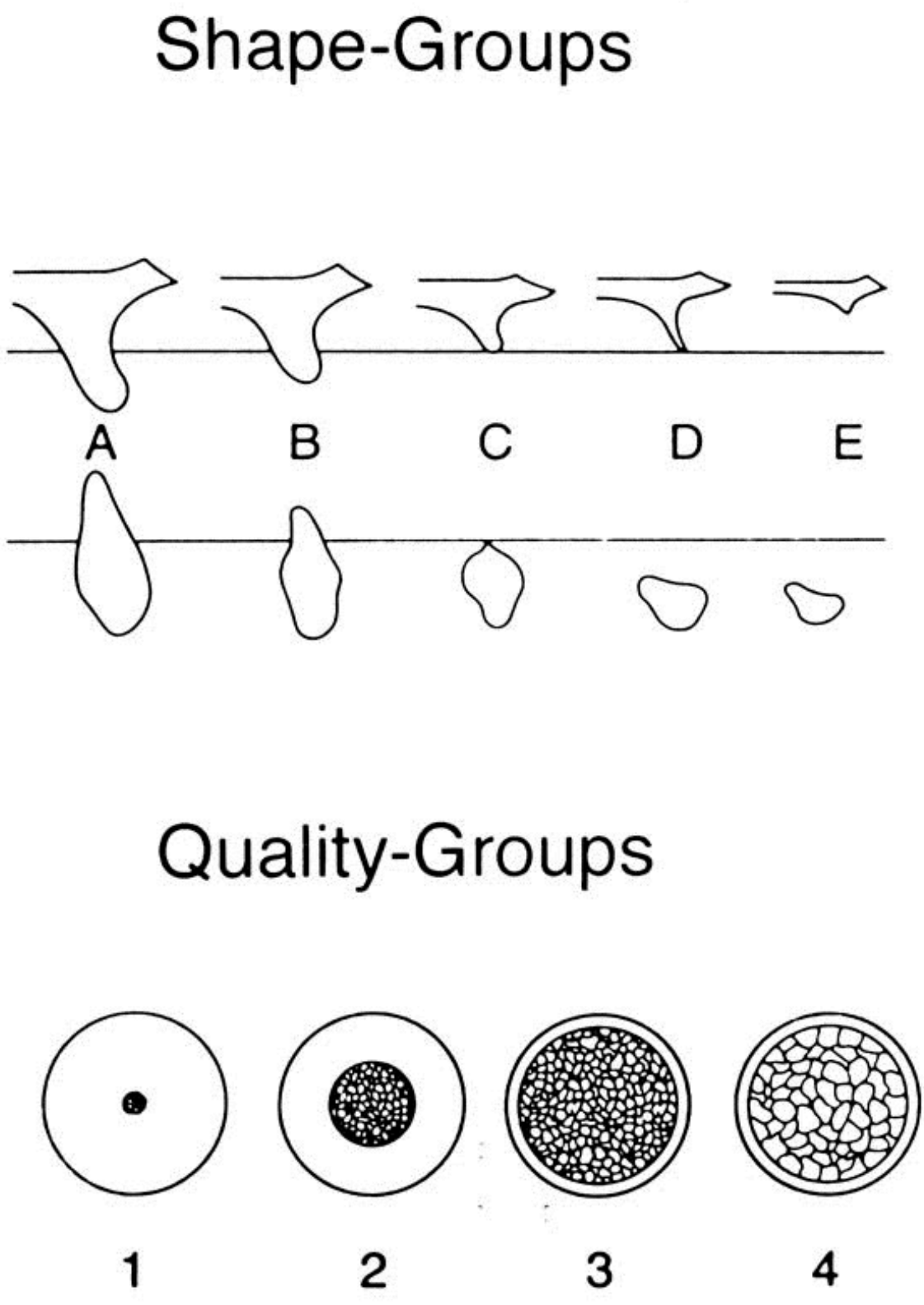

Figure 1.6: Classification of bone quality and jaw shape. Reproduced with permission. (105).

Several longitudinal studies on implant failure rates reports increased failure risk for implants placed in type 4 bone locations(106, 107). Increased failures in type 4 bone are important because patients affected with osteoporosis classically have type 4 bone due to loss of bone mineral density(108). With controversy in the literature, histologic studies demonstrated no difference in bone to implant contact (BIC) among healthy and osteoporotic patients (109). However, a large retrospective study concluded that osteoporosis was a significant variable for early implant failure(7).

Currently, the role of anti-resorptive medications is still unclear on their effects of implant outcomes(7). The first reports of osteonecrosis of the jaw due to anti-resorptive medications, (109, 110) specifically bisphosphonates, was in 2003(111). Monoclonal antibodies gained popularity to aid in treating osteoporosis and other skeletal metabolic and/or metastases as they were thought to have fewer side effects, however, the first report of ONJ related to these types of medications was reported in 2010(112). Due to the increased number of cases, and their effect on bone metabolism, there has been an increase in the number of publications on bisphosphonates and other anti-resorptive drugs related to osteonecrosis of the jaw(113, 114). Bisphosphonates are medications used to treat a variety of skeletal disorders, ranging from metabolic bone conditions to metastatic bone diseases, resulting in the maintenance of bone density and maintenance in serum calcium levels(62, 115). The prevalence of ONJ varies depending upon route of administration, condition being treated, length of use, and population, ranging from <1% to as high as 28%(116, 117). Because ONJ appears to be localized to the oral cavity, surgical dental interventions may put patients at an increased risk for the development of ONJ, including extractions and possibly dental implants during or prior to the initiation of anti-resorptive therapy(117). Currently,

there is little solid epidemiological evidence supporting compromised outcomes of implant therapy in patients treated with anti-resorptive medications. Compromised outcomes in dental implant therapy due to anti-resorptive mediations can have an extensive impact as millions of patients, as both men and women with osteoporosis or other metabolic bone disorder are taking these medications.

Overall opinion in the field is more research is needed to ascertain if a true correlation exists between alveolar changes in the maxillofacial region and overall skeletal bone mass(118). Also, additional research will be able to evaluate if medications used to treat osteoporosis have effects on implant outcomes.

Section 1.10: Porous Tantalum Trabecular Metal: Production and Biologic Influence

Porous tantalum trabecular metal (PTTM) is made up of a rare transitional metal, Tantalum (Ta), and is known for its resistance to corrosion. It is a member of the refractory metal groups, allowing it to be incorporated in various alloys(119). Anders Gustav Ekebereg, a Swedish chemist discovered Ta in 1802(120). Ta is predominantly mined in western Australia and extracted from tantalite, but can be produced as a by product of tin mining(121). Previously, Ta had restricted applications in the medical field due to its rarity and the difficulty in manipulating solid Ta(119). Ta is overly reactive to oxygen, however, due to this exaggerated reactivity, the formation of oxides on the surface is immediate when exposed to oxygen, rendering the surface inert(119). Its use in the medical field includes orthopedic implants, electrodes for pacemakers, and devices for nerve repair(122, 123). Ta was incorporated in orthopedic implants to mimic the natural structure of trabecular bone. PTTM has a structure similar to trabecular bone through the use of repeating dodecahedron three dimensional repeats(124). The structural morphology is constructed through the use of

thermosetting foam polymer foam, which has undergone pyrolysis, thus creating a vitreous carbon scaffold(125). Tantalum is then deposited on the surface of the scaffold through the use of vapor deposition and infiltration(126). Due to the microarchitecture of PTTM it has a low modulus of elasticity, which is similar to that of cancellous bone, leading to more ideal dispersion of load and a reduced stress shielding phenomenon(124, 127).

The porous structure of PTTM has a biologic impact through the induction of rapid angiogenesis by adhesion of serum proteins, leading to recruitment of osteoblastic precursor cells and subsequent matrix formation(128). PTTM has a higher degree of porosity than traditional titanium implants(124). Orthopedic surgeons have long utilized porous tantalum trabecular metal (PTTM) for its ability to enhance peri-implant wound healing. The use of PTTM in orthopedic implants over the years has shown excellent vascularization, bone ingrowth, osteoconductivity and biocompatibility(125, 129). Tantalum has been shown to be bioactive by forming a bone-like apatite layer when exposed to body fluid, and biologically binds to bone(130, 131). The porous structure provides a biologically similar scaffold shape to trabecular bone and allows for the bone in-growth and mechanical attachment to implant surfaces(125, 132). The bone ingrowth due to the porous structure has lead to the concept known as “osseoincorporation”(124, 125).

Summary

Throughout the history of implant therapy, the goal has been the formation and maintenance of osseointegration, a direct connection between the hard tissues of the jaw and the implant surface. With the evolution of macrostructure, micro- and eventually nanotopography, osseointegration has become increasingly predictable, even in patients with systemic conditions(133). Osseointegration is not without failures; in some cases

there are local and systemic factors play a role in the formation and maintenance of this structural and functional relationship between the implant surface and bone(7, 97). Once implant success became more predictable, the focus then shifted to hastening the process and inducing a more vigorous osseointegration response in healthy and patients predisposed to implant failure. The introduction of porous tantalum trabecular metal (PTTM) was utilized during orthopedic implants as an alternative to titanium due to its biologic response and ability to be formed in porous three-dimensional open cell structures to facilitate enhanced bone ingrowth(124, 132). The designed porosity allows for its enhanced osteoconduction and angiogenesis, permitting bone to actually anchor onto the outer surface and inside the interconnected pores of PTTM, characterized as "osseoincorporation"(119, 130, 134, 135). PTTM has recently crossed over to use in the oral cavity to replicate the hard tissue response seen in orthopedic implants(119, 136). Tantalum was selected as an alternative to titanium due to its modulus of elasticity that is similar to trabecular bone and its resistance to corrosion coupled with improved frictional properties(121, 125, 126). However, it is similar to titanium with concern to the biocompatibility, biochemical and biomechanical properties that support osseointegration(125, 126). What continues to warrant further investigation is whether PTTM implants are able to more robustly induce osseointegration in patients with risk factors that compromise bone remodeling.

CHAPTER 2: OSTEOGENIC ACTIVITY ASSOCIATED WITH DENTAL IMPLANT PLACEMENT IN PATIENTS WITH OSTEOPENIA/OSTEOPOROSIS AS COMPARED TO HEALTHY INDIVIDUALS

Introduction

The implementation of dental implants for the treatment of partial and fully edentulous patients has evolved through the years and become common practice throughout the world. Dental implant therapy is considered an effective, safe and reliable method of treatment that is a viable alternative to conventional fixed and removable prostheses. Regardless of the documented predictability, failures still occur and patients with certain behavioral and systemic conditions are at increased risk of failure(8). The predictability of dental implant therapy is predicated on the ability to achieve and maintain intimate contact with the alveolar bone that is both a functional and structural relationship, known as osseointegration(137, 138). The gold standard for determining the success and degree of osseointegration is histology, however, this is not a viable option clinical practice(139). Therefore, the success of osseointegration has been defined by a lack of increasing mobility between the implant and the surrounding trabecular bone after implant placement(83).

Throughout the years, research has been done to investigate the complex pathways involved in bone healing *in vivo*, however the minutia involved in these signaling pathways has not been fully elucidated(140). While research has teased apart the major pathways involved in osseointegration of healthy patients, even less is known about

intricate interaction of pathways involved in osseointegration in vivo medically compromised patients(22, 141).

With the introduction of new biomaterials, such as PTTM, their ability to aid in more efficient osseointegration, not only in healthy but medically compromised patients is currently being researched(142).

Section 2.1 Methods and Materials

Clinical Relevance

Porous tantalum trabecular metal (PTTM) may enhance initial implant healing in the oral cavity as shown in orthopedic implant studies and therefore may be indicated for early implant loading and restoration in healthy subjects. PTTM may also be useful for subjects with compromised bone healing or density; however, future studies are needed for compromised healing population. The primary aim of the study was to examine whether or not the PTTM can improve bone ingrowth, thereby increasing the bone to implant contact (BIC). It was theorized that the micro porous structure would induce earlier bone deposition around implants when compared to conventional titanium (Ti) alloy, leading to more robust osseointegration.

Participants

This study was approved by the Biomedical Institutional Review Board of the University of North Carolina at Chapel Hill, IRB:11-2539. Written informed consent was obtained from all study participants. The study population consisted of 13 adults in adequate periodontal health that were eligible and treatment-planned to receive mandibular dental implants. One subject was withdrawn due to insufficient alveolar bone. Subjects with

symmetrical edentulous areas in the mandible, requiring placement of at least 4 implants, were recruited through UNC and its healthcare system. The control group for the histologic arm of the study consisted of 6 systemically healthy individuals, and the experimental group consisted of 6 patients with osteoporosis, diagnosed previously by a physician. Major exclusion criteria included: use of medications known to affect periodontal status within one month prior to initial examination, systemic conditions that are known to affect periodontal status, history of IV bisphosphonates, active infectious disease, pregnancy, current smokers or history of smoking within the last two years, subjects with blood disorders and/or anticoagulant therapy, chemotherapy, and radiotherapy. Specific inclusion and exclusion criteria are shown in Figure 2.1.

Inclusion Criteria

- Subjects must be adult males or females between the age of 18 and 80 years (inclusive).
- Subjects must be able and willing to follow study procedures and instructions in English.
- Subjects must have read, understood and signed an informed consent form in English
- Subjects must have at least two mandibular implants as their future treatment needs.
- Subjects must meet one of the following categories to be considered for enrollment:

Exclusion Criteria

- Individuals who have a chronic disease with oral manifestations
- Individuals who exhibit gross oral pathology
- The use of either antibiotics or chronic use of NSAIDS within 1 month prior to screening examination
- Individuals that require antibiotic prophylaxis prior to dental treatment
- Chronic treatment (i.e. two weeks or more) with any medication known to affect periodontal status (e.g. phenytoin, calcium antagonists, cyclosporine, Coumadin) within 1 month prior to screening examination
- Systemic conditions, except osteoporosis and osteopenia that are known to affect periodontal status
- Individual with uncontrolled parafunctional habits, such as clenching and bruxing on objects, that could adversely impact implant survival
- Individuals with a history of intravenous bisphosphonates, individuals with active infections diseases such as hepatitis, HIV or tuberculosis
- Individuals with a current tobacco use history
- Individuals who are pregnant, breastfeeding, or planning to become pregnant within 3 months

Osteoporosisis or osteopenia patient Inclusion criteria:

- Subjects must be diagnosed with osteoporosis or osteopenia and must be currently under the care of a physician and treatment with oral bisphosphonates. Subjects must have never had intravenous (IV) bisphosphonates. Subjects in this group must be non-diabetic and no history of smoking within the last 2 years

Figure 2.1 Inclusion and Exclusion Criteria

The included subjects' demographic information is shown in Table 2.1.

Demographics	Healthy	Osteopenia/ Osteoporosis
N=12	6	6
Age	64.3±6.0	66.38±5.15
Male	4	0
Female	2	6
Caucasian	5	5
African American	1	1
Cross Sectional Processing	2	3
Longitudinal Processing	4	3

Table 2.1: Patient Demographics

An edentulous ridge area with sufficient space to place two test cylinders (each approximately 2.9-3 x 5 mm) was confirmed radiographically. Patients had a CBCT taken with a radiographic stent to confirm adequate alveolar dimension. For each patient, two titanium test cylinders were placed on the mandible at the level of the crestal bone and covered with a collagen membrane (BioMend, Zimmer Dental, Carlsbad, CA, USA) to prevent soft tissue down growth, primary closure was obtained. Test cylinders were removed at two and four weeks using a 5.0 mm diameter tissue punch and 4.5mm trephine drill and sites received a screw vent implant (Zimmer Dental, Carlsbad, CA, USA), upon the removal of the test cylinder.

Study design

Each subject was screened clinically and radiographically. Cone-beam computed tomography (CBCT) scans were used to examine the pre-existing alveolar bone. The CBCT scans were reviewed by one of the implant surgeon investigators (SB or TM). Simplant 16 (Dentsply, York, PA) or 360dps implant planning software (360imaging, Atlanta, GA) was used to determine if there was sufficient bone for future dental implant therapy. A minimal width of 7mm and height of 8mm for each dental implant was required for inclusion into the study. The minimal bone volume was needed to ensure that the 3mm x 5mm diameter device could be placed, preventing bone dehiscence or approximating any anatomical structures. At each surgical visit, subjects were asked to rinse their mouth with 0.12% Chlorhexidine gluconate prior to any procedure. All surgical procedures were completed under local anesthetics involving bilateral inferior alveolar nerve blocks, long buccal nerve blocks and local infiltration of the surgical sites. Full thickness flaps were raised in all sites. A split-mouth design was used. Four osteotomy sites were located and prepared based on the pre-operative

CBCT scans, implant planning software, and a surgical guide. Two 3mm x 5mm Ti alloy tapered screw and two 3mm x 5mm PTTM cylinder devices were placed in each edentulous site, Ti test cylinders on one edentulous side of the jaw and PTTM test cylinders on the other as seen in Figure 2.2 and 2.3.

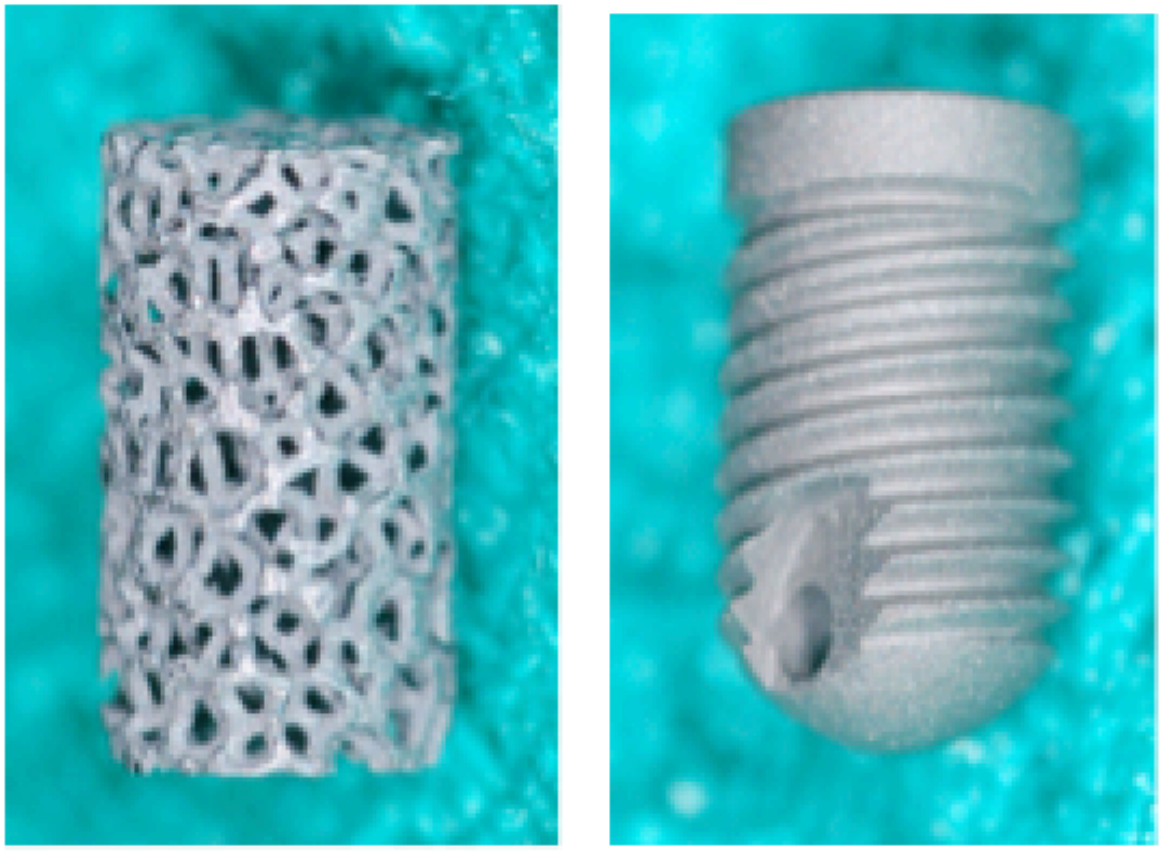

Figure 2.2: Ti and PTTM Test Cylinders. (Photo courtesy of Dr. Thiago Morelli).

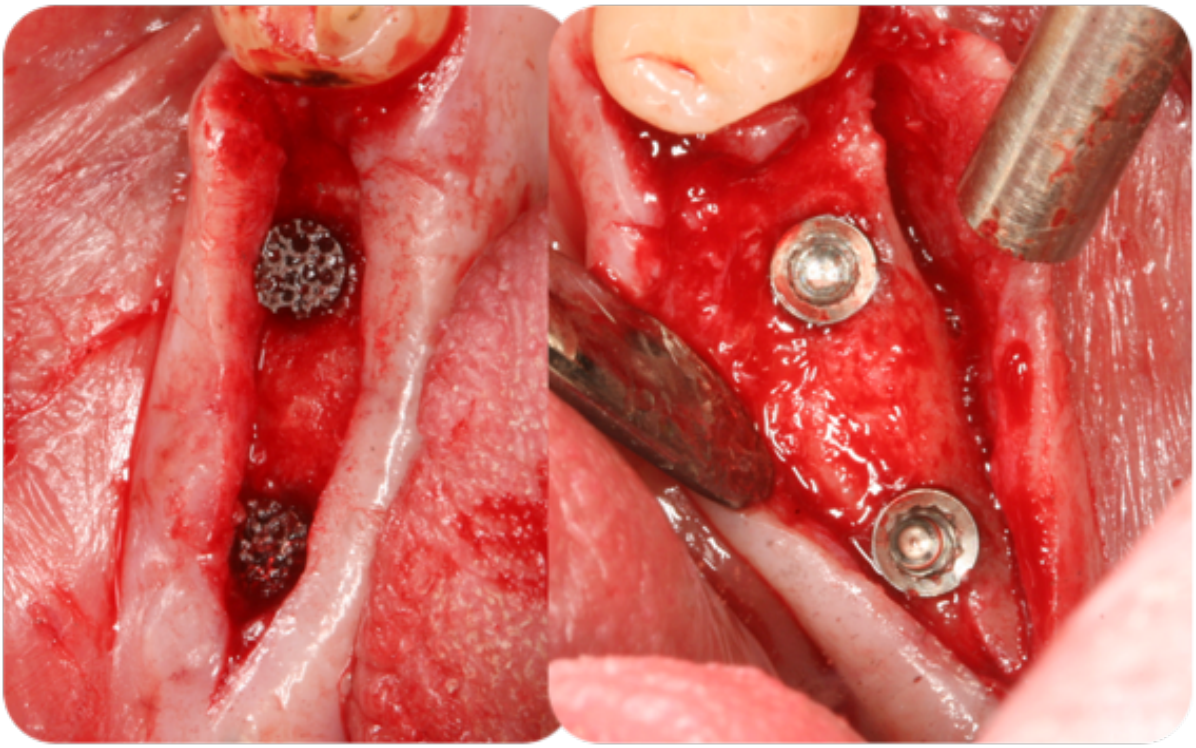

Figure 2.3: Test cylinders immediately after insertion, right photo is PTTM test cylinders, left photo is Ti test cylinders in the same patient. (Photo courtesy of Dr. Thiago Morelli).

The top of each test cylinder was placed at the level of crestal bone level to ensure that the device was completely surrounded by bone. Note that since the Ti device was a self-tapping tapered screw, a 2.3 mm drill was used and the Ti device was self-tapped in place. Ti device also has HA blasted surface treatment mimicking the clinically used and commercially available dental implants (MTX, Zimmer Biomet, Palm Beach Garden, FL). However, since the PTTM device was a straight cylinder, a 3 mm drill was used to place the device. The PTTM device was press-fitted in place. A 5 mm diameter resorbable collagen membrane (Biomend, Zimmer Biomet, Warsaw, IN) was placed on top of each device. A combination of continuous interlocking and interrupted suturing techniques with 4.0 chromic gut sutures were used to ensure the primary closure and hemostasis. The subjects were instructed to continue using a 0.12% Chlorhexidine rinse until the completion of the study (a total of about

6 weeks, 2 weeks more after the final surgery). One device from each group was removed using 4.5 mm diameter trephine drill (Salvin Dental Specialties, Charlotte, NC). One device was from each metal type (PTTM or Ti) were removed at two weeks for non-histological analysis (data not reported as part of this thesis). Remaining test cylinder samples were explanted at the 4-week visit for histological analysis from both the healthy and osteopenia patient samples for both metal types (PTTM or Ti). Histology was only evaluated in half of the samples collected at 4 weeks. Histological analysis was only completed at the 4 week time point as any early, such as the 2 week time point would not have yielded sufficient mineralized tissue. The explantation, through the use of the trephine, was completed in conjunction with a surgical guide, individually fabricated for each patient, the surgical guide with trephine can be seen in figure 2.4. After explantation, each cylinder was placed separately into a microfuge tube containing 4% paraformaldehyde. Test cylinders used for histologic analysis were processed properly (sample processing is described below).

Figure 2.4: Trephine with stent. (Photo courtesy of Dr. Thiago Morelli).

After each device removal, the osteotomy site was prepared for the definitive implant. Conventional titanium Tapered Screw Vent (TSV) implants (Zimmer Biomet, Warsaw, IN) were placed at each site. Bone allograft, Puros Cancellous Particulate (Zimmer Biomet, Warsaw, IN), and appropriate resorbable collagen membrane such as Biomend (Zimmer Biomet, Warsaw, IN) were used as needed if there was bone dehiscence around the dental implant. The subjects were then seen at least one more time 2 weeks after the removal of the final devices and placement of definitive dental implants. After the clinical operator determined that the subjects had appropriate soft tissue healing, subjects then exited the study and were referred back to their restorative providers to fabricate appropriate definitive prostheses.

Histology

Raw histologic samples comprised of tissue blocks from the test cylinders were prepared for ground sectioning utilizing the following methods. After explantation, each cylinder was placed separately into a microfuge tube containing 4% paraformaldehyde, then transferred to 0.1M Cacodylate buffer, pH 7.4, for several hours to overnight. Dehydration was started with an ethanol series: 50%, 70%, 95% ethanol in distilled water for 10 min each. They were then transferred into absolute ethanol for two rinses of 20 min each. The samples were infiltrated with a 50:50 mixture of Polybed resin (Polysciences Inc, Warrenton, PA) and absolute ethanol for 6-12 hours. They were then embedded with several changes of pure resin into BEEM® capsules and cured overnight at 65 degrees Celsius. The orientation of the samples during embedment was carefully maintained to facilitate cross-sectional or longitudinal slicing of the implant samples. Cured resin blocks containing the implants were removed from the polyethylene capsules and were sectioned following the long axis or short axis of the implants using a Buehler diamond band saw fitted in a precision slicing machine (IsoMet® Low Speed Saw; Buehler; Lake Bluff, IL) with a thickness of approximately 50–60μm. Histological slides were stained with toluidine blue and examined under confocal microscope (Olympus® IX81) using bright field (BF) technique. Histological images were taken using image analysis software (Volocity; PerkinElmer®, Waltham, MA) taking stacks of images up to 30 separately focused stacks for each slice. Images were taken at a magnification of 4x and 20x. Due to the three dimensional structure of the trabecular test cylinders that required the use of image stacking, an image processing program was utilized to stitch stacked images together utilizing the software ImageJ (developed by the NIH). The use of digital refocusing with extended depth of field (EDF) plugin was utilized to complete

and merge focus stacking from different focal positions, providing an entirely focused composite image. Composite images were then analyzed in ImageJ to calculate BIC percentage.

Histologic samples were randomly assigned to be processed in one of two methods, cross sectional or longitudinal processing. A total of 6 patients, 4 healthy control and 2 experimental (osteopenia/osteoporosis) were sectioned longitudinally and analyzed for surface area, BIC percentage and total BIC.

Section 2.2 Statistical Analysis

A general linear model (GLM); a type of ANOVA, utilizing a least squares regression approach to analyze both BIC percentage and BIC total comparing the different combinations of metal (titanium and PTTM) and patient type (healthy and osteopenia/osteoporosis), utilizing SAS software. A General estimating equations (GEE) model was used to control for the repeated measures within subject at one time point for the different metal types (Ti or PTTM). Level of significance was set at 0.05.

Differences within individuals patients also know as change scores were analyzed using a T-test within subject to analyze the BIC percentage change and the total BIC for the different test cylinder metal types (PTTM vs Ti). Level of significance was set at 0.05.

Section 2.3 Results

Four osteopenic/osteoporotic female subjects aged between 59 to 76 years were included in this study. Six healthy male and female subjects aged 54 to 71 were included in this study. A total of 20 experimental cylinders (n = 10 Ti control cylinders and 10 PTTM

test cylinders) were placed. Each subject received 2 adjacent test cylinders and 2 adjacent control cylinders on opposites sides of the same jaw. At each time point, one Ti and one PTTM test cylinder were retrieved from each subject. Cylinders retrieved at the 4-week time point were randomly selected for histologic evaluation or genetic profiling. Comparisons were performed to analyze PTTM cylinders to the Ti cylinders in osteopenic/osteoporotic patients at 4-week time points as well as osteopenia/osteoporosis patients to healthy control patients.

Histology

Histological evaluation was the method used to determine if there was increased BIC between the two materials/microstructures. Longitudinal and cross sectional analyses were performed for subjects in the control and experimental groups. Longitudinal sectioning was utilized to calculate the BIC percentage, total and surface area, while cross sectional was utilized to qualitatively evaluate the bone ingrowth and penetration into the PTTM. While cross sectional slicing was utilized to qualitatively evaluate the bone ingrowth and penetration into the PTTM test cylinders in direct comparison to the Ti test cylinders. Comparison of 4x magnification of Healthy (Ti and PTTM) to Osteopenia/Osteoporosis (Ti and Ta) 4x magnification was completed and analyzed, Figure 2.5 and Figure 2.7. The BIC total and BIC percentages were not calculated for the cross sectional histological slices. BIC was calculated as a percentage and as a total, both of which were analyzed for statistical significance. Two patients from the experimental (Osteoporosis/osteopenia group) and two patients from the control (healthy group) were utilized for cross sectional qualitative evaluation.

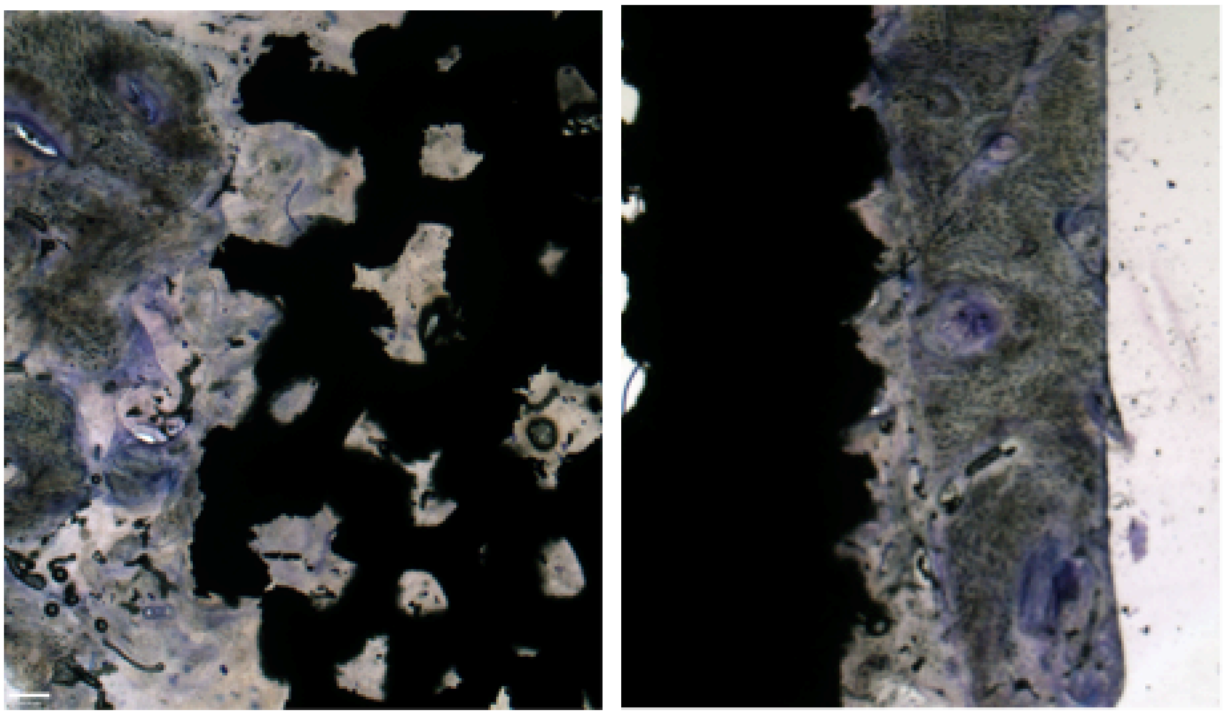

Figure 2.5: Histology 4x Tantalum (a) and Titanium (b) test cylinders in healthy patients.

Comparison of 20x magnification of Healthy (Ti and Ta) to Osteopenia/Osteoporosis (Ti and Ta) 20x magnification was completed and analyzed, Figure 2.6 and Figure 2.8.

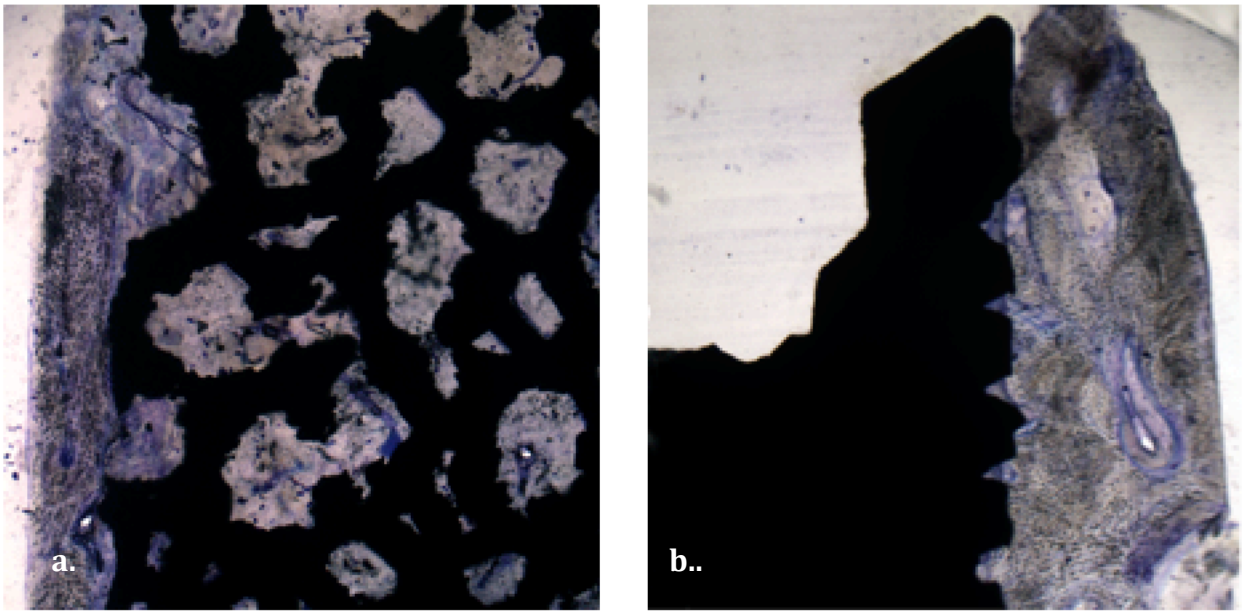

Figure 2.6: Histology 4x Tantalum (a) and Titanium (b) test cylinders in osteonpenia/osteoporotic patients.

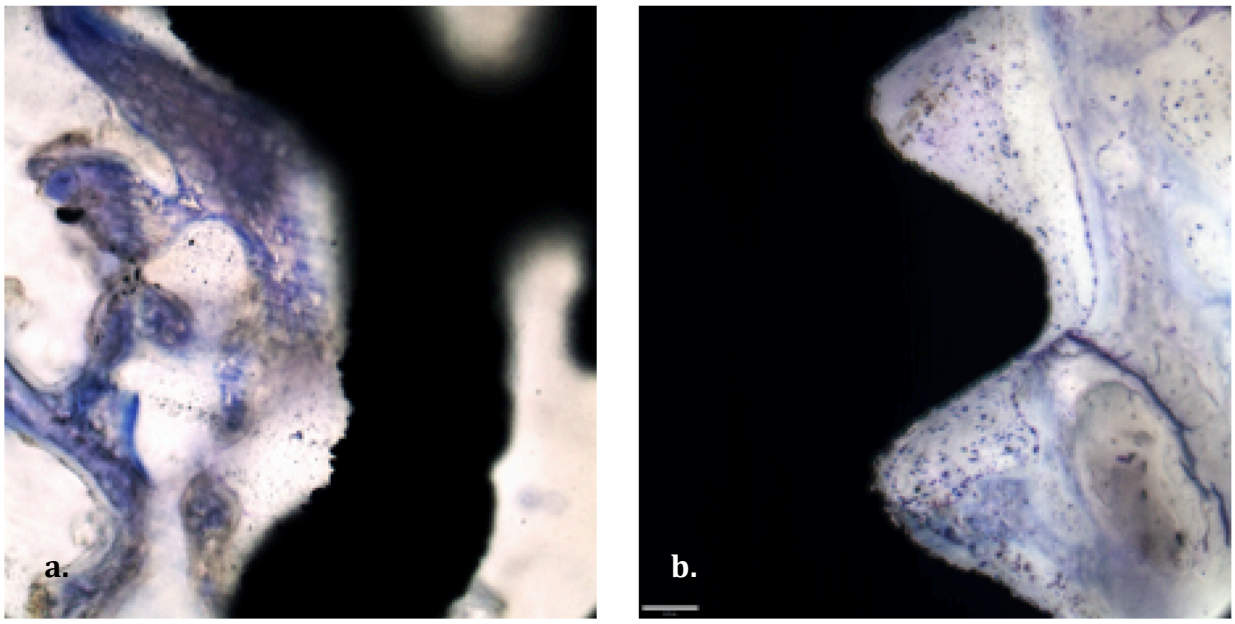

Figure 2.7: Histology 20x Tantalum (a) and Titanium (b) test cylinders in healthy patients.

Cross sectional cuts were made from selected subjects to visualize the depth of bone penetration. Examples of the cross sectional comparison at 4x is seen in Figure 2.9.

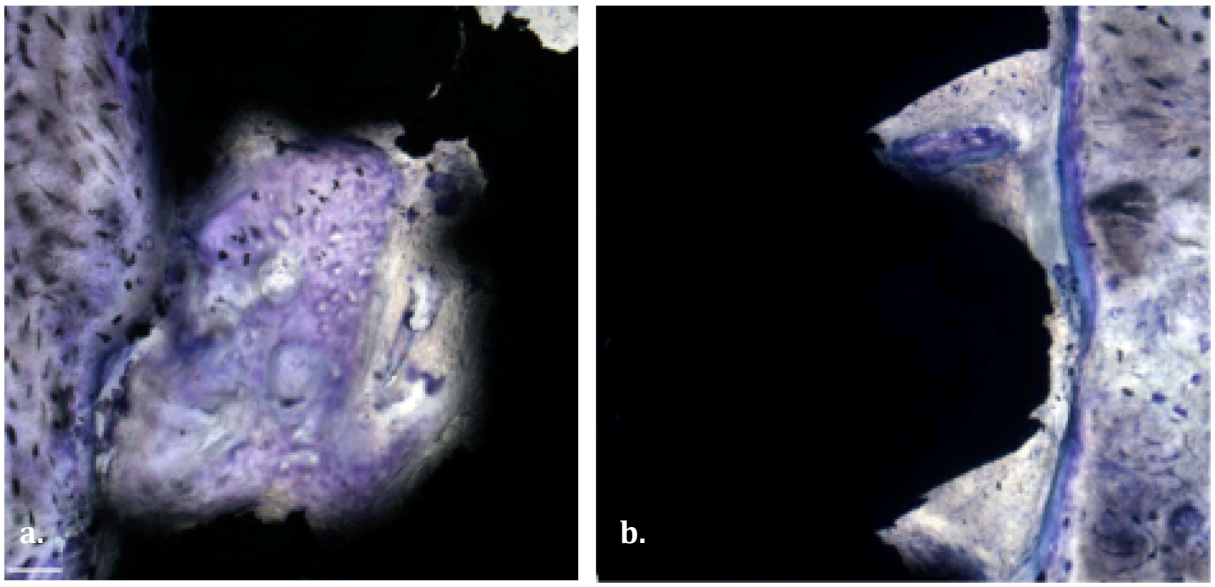

Figure 2.8: Histology 20x Tantalum (a) and Titanium (b) test cylinders of osteopenia/osteoporotic patients.

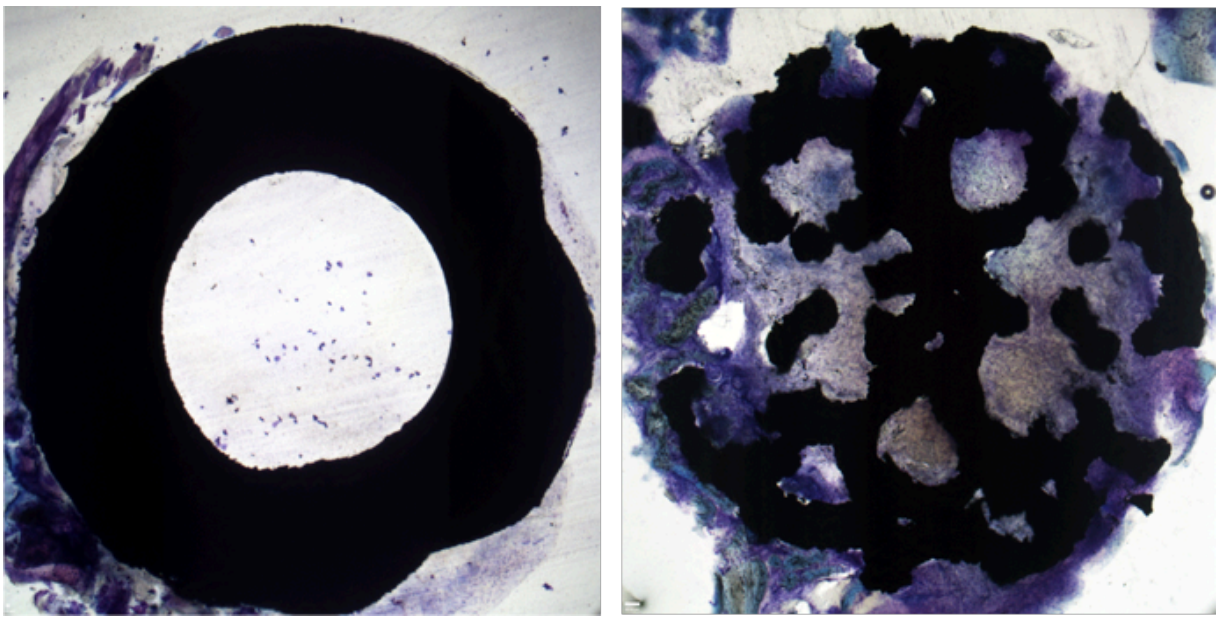

Figure 2.9: Cross-sectional slices of Titanium (left), and PTTM (right) test cylinders.

Graphical representation of statistical comparison of mean BIC percentage can be seen in. Figure 2.9 and Table 2.2 and 2.3, while mean total BIC can be seen in Figure 2.11 and Table 2.4 and 2.5. BIC was calculated as a percentage, by calculating the surface area of test cylinder for the selected field of view, then the bone in direct contact with the implant was calculated and a percentage was obtained. The total BIC was calculated based on the total bone in contact with the test cylinder surface, irrespective of surface area. All calculations utilized the longitudinal samples for quantitative analysis, however, the cross sectional slices were utilized for qualitative evaluation.

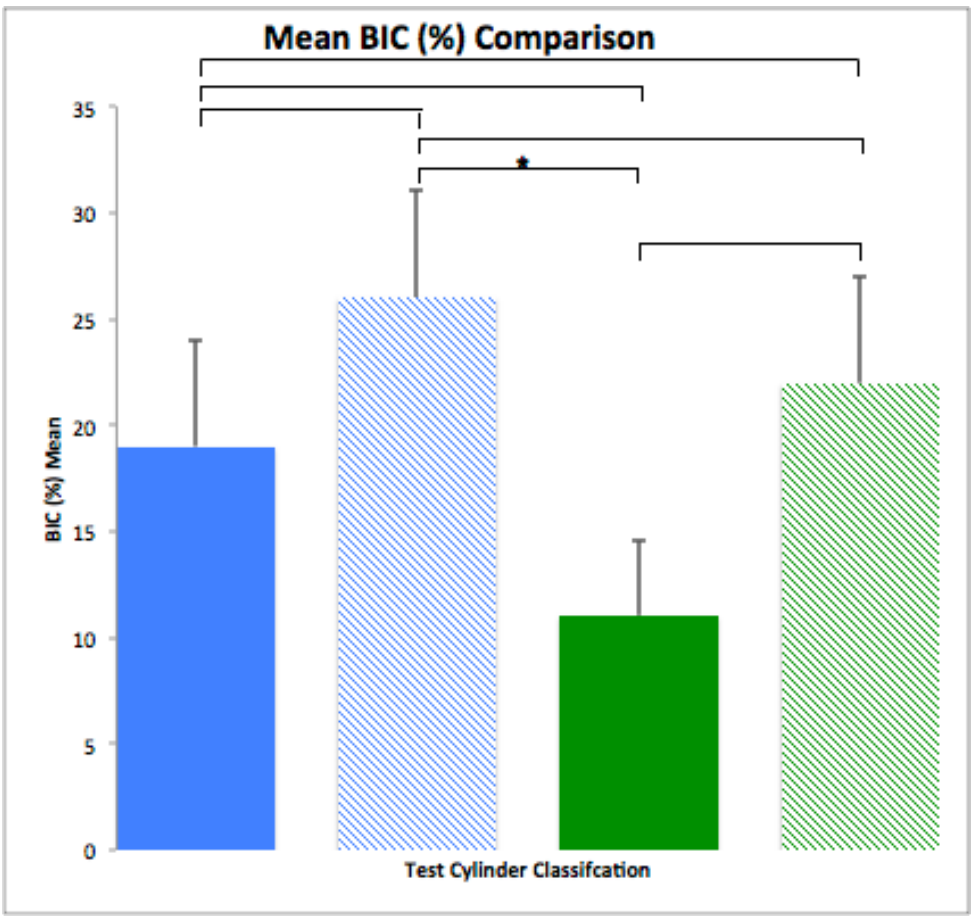

Figure 2.10: GLM Analysis of BIC mean percentage (%). Comparison completed between healthy and osteopenia/osteoporosis and titanium and tantalum. Solid colors represent healthy, diagonal lines represent osteopenia/osteoporosis. Blue represents Titanium and green represents PTTM. * represents statistical significance (P= <0.05)

Group Type	Metal Type	BIC Mean Percentage	Standard Error
Healthy	Titanium	19.0	±5.0
Healthy	Trabecular (PTTM)	11.0	±3.5
Osteopenia	Titanium	26.0	±5.0
Osteopenia	Trabecular (PTTM)	21.9	±5.0

Table 2.2: GLM Analysis of BIC mean percentage (%). Comparison completed between healthy and osteopenia/osteoporosis and titanium and tantalum. Level of significance set at (P= <0.05). Statistical significance found comparing Osteopenia/Osteoporosis titanium to Healthy trabecular subjects. No other statistical difference was noted.

Parameter	Estimate	Standard Error	95% Confidence Limits	P Value
Titanium	5.9	1.5	2.9 - 8.8	0.001
Osteopenia	10.9	3.4	4.3 - 17.6	0.0012
Osteopenia Titanium	-1.8	1.4	-4.9 - 1.1	0.23

Table 2.3: Analysis of GEE Parameter Estimates for Mean BIC Percentage, Empirical Standard Error Estimates.

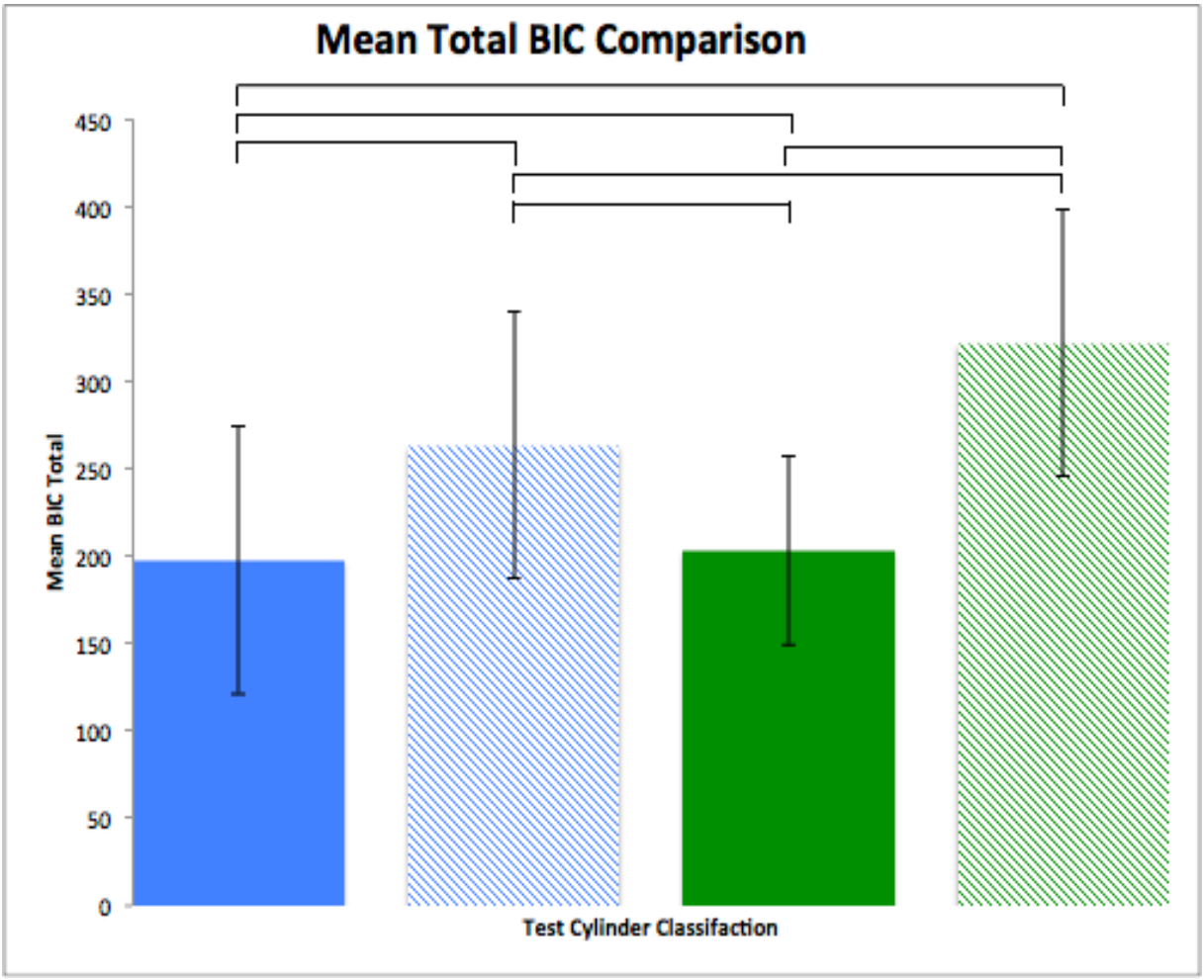

Figure 2.11: GLM Analysis of BIC mean total. Comparison completed between healthy and osteopenia/osteoporosis and titanium and tantalum. Solid colors represent healthy, diagonal lines represent osteopenia/osteoporosis. Blue represents Titanium and green represents PTTM

Group Type	Metal Type	BIC Mean Total	Standard Error
Healthy	**Titanium**	**197.5**	**±76.4**
Healthy	**Trabecular (PTTM)**	**203.2**	**±54.0**
Osteopenia	**Titanium**	**263.2**	**±76.4**
Osteopenia	**Trabecular (PTTM)**	**321.6**	**±76.4**

Table 2.4: GLM Analysis of BIC mean total. Comparison completed between healthy and osteopenia/osteoporosis and titanium and tantalum.

Parameter	Estimate	Standard Error	95% Confidence	P Value
Titanium	-58.5	28.5	-114.3 - -2.6	0.04
Osteopenia	65.7	94.2	-118.9 - 250.4	.4856
Osteopenia Titanium	0.07	31.8	-62.2 - 62.4	0.9

Table 2.5: Analysis of GEE Parameter Estimates for Mean Total BIC, Empirical Standard Error Estimates.

The difference within patient samples, comparing the different metals was calculated using a change score and analyzed for significance utilizing a t-test. Mean difference for BIC percentage, Total BIC and total surface area were calculated. Mean difference BIC

Percentage can be seen in Figure 2.12. While mean difference total BIC can be seen in figure 2.13 and mean difference of surface area can be seen in figure 2.14.

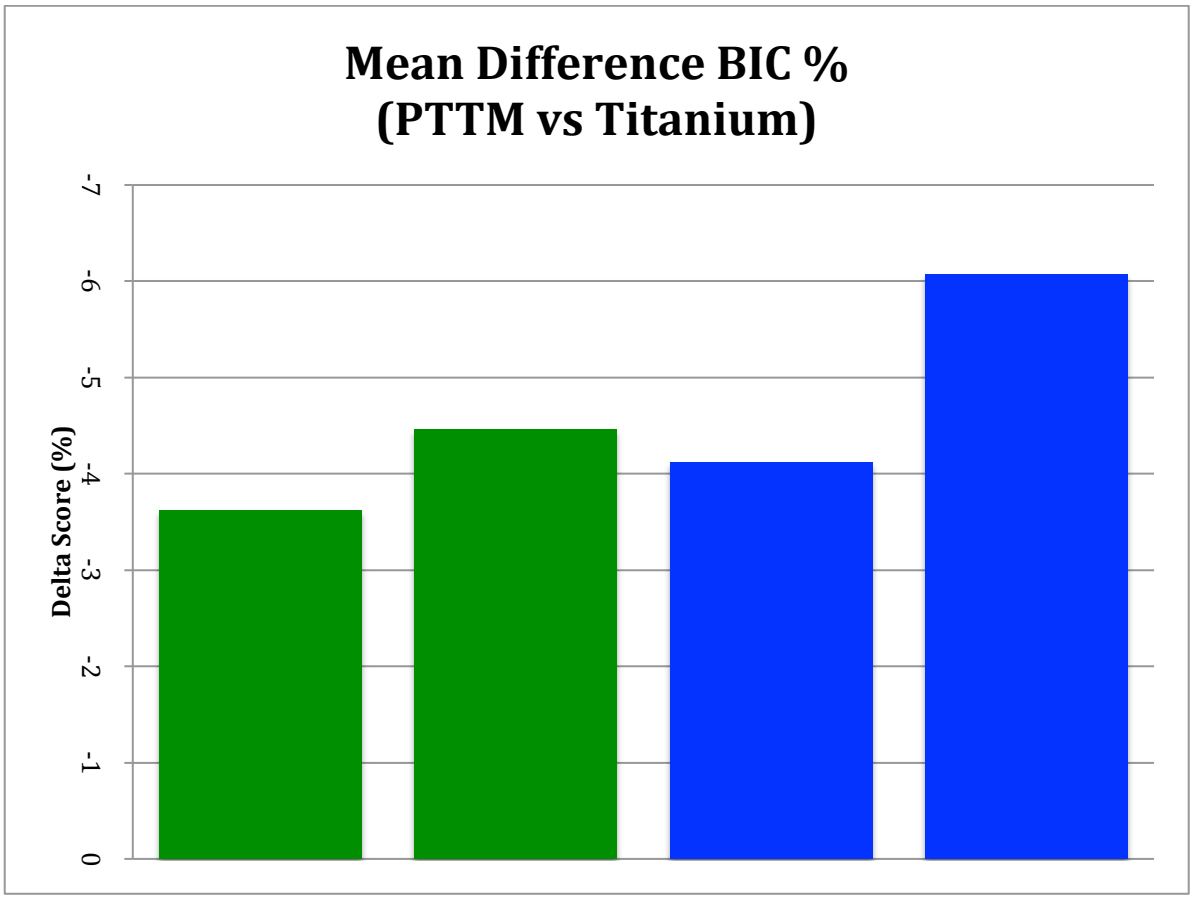

Figure 2.12: Mean difference in BIC percentage within individual patients comparing PTTM vs Ti. Green is osteopenia, blue is healthy.

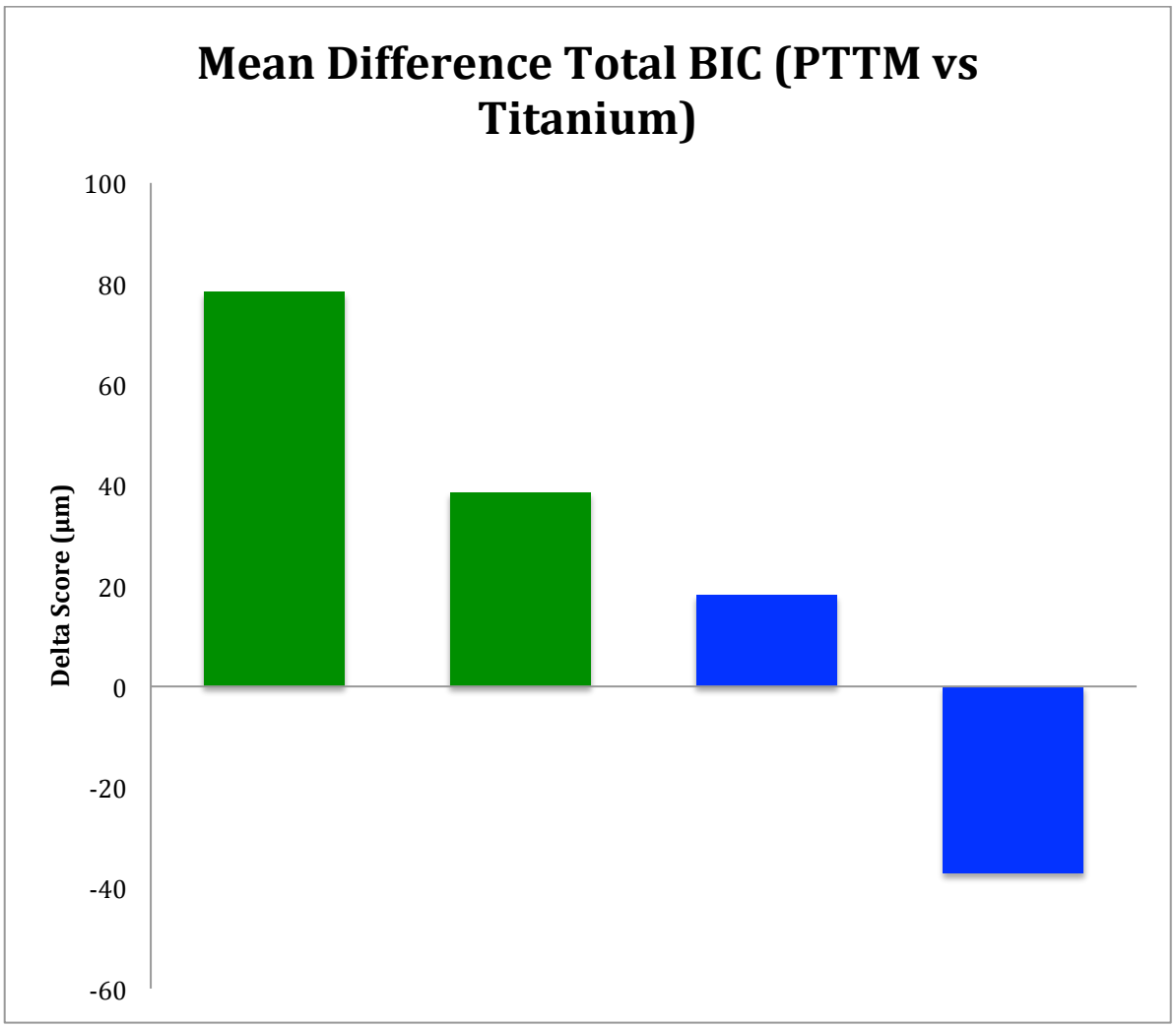

Figure 2.13: Mean difference of total BIC within individual patients comparing PTTM vs Ti. Green is osteopenia, blue is healthy

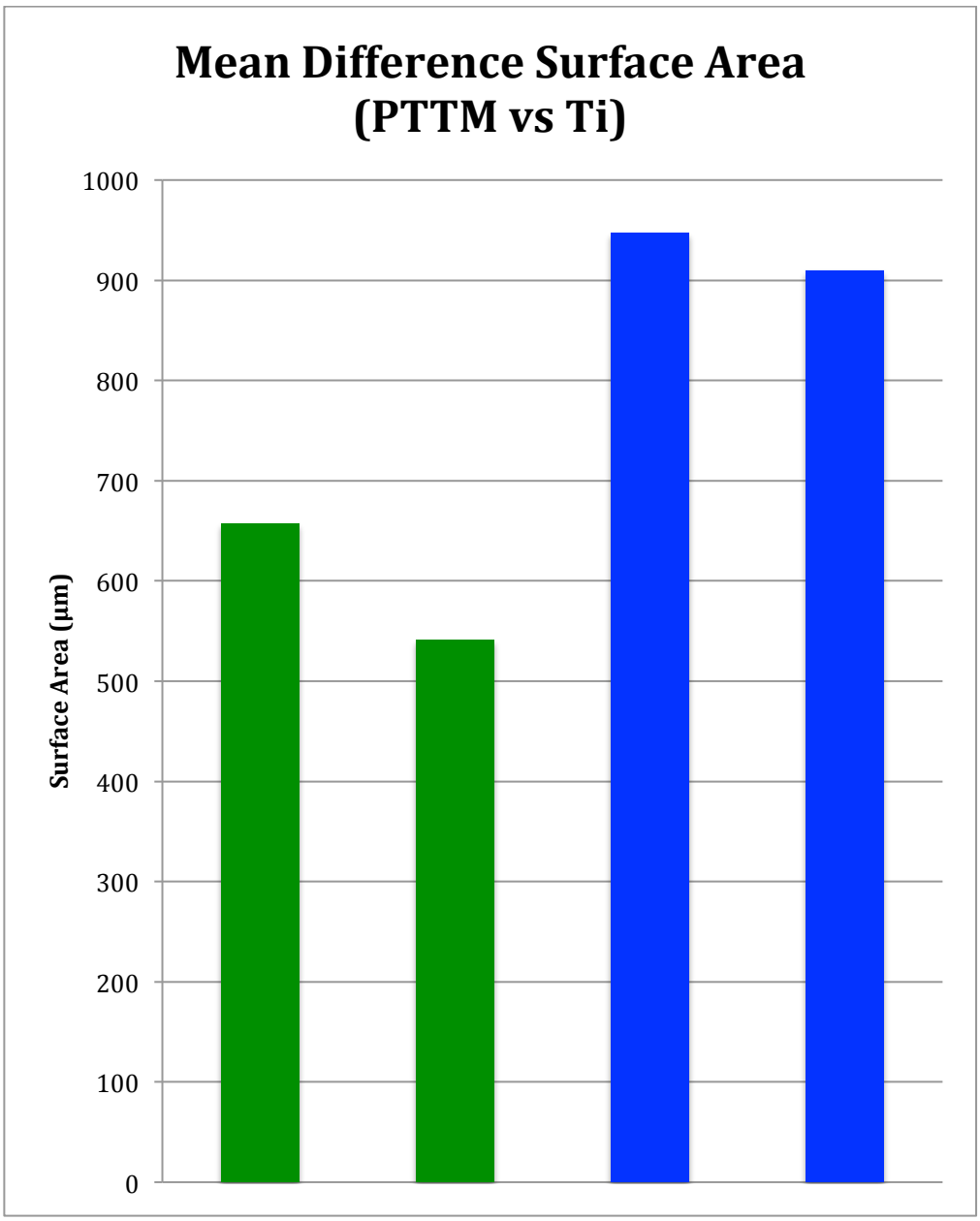

Figure 2.14: Mean difference of total surface area within individual patients comparing PTTM vs Ti. Green is osteopenia, blue is healthy

There was not a statistically significant difference between the titanium or PTTM test cylinders for either group (healthy or osteopenia/osteoporosis) of patients, when comparing means for BIC percentage, total BIC or surface area P value= >0.05. A trend was seen during the analysis of the surface area in favor of the PTTM, however, no statistical

difference was noted between the healthy vs experimental groups. No statistical difference was found between the experimental or healthy group, or between the titanium and PTTM group except when comparing the healthy titanium to the osteopenia/osteoporosis PTTM group, P=<0.05. With the GEE model for the BIC percentage comparison, Ti test cylinders showed a significantly higher percentage of BIC compare to PTTM test cylinders, in addition, osteopenia/osteoporosis patients showed a significantly higher percentage of bone to implant contact compared to healthy patients. Once again the interaction between metal type and disease/health status was not significant. Utilizing the GEE model for BIC total comparison, Ti test cylinders showed a significantly reduced amount of total bone contact compared to PTTM test cylinders, while Osteopenia/osteoporosis patients showed and increased amount of total bone contact with either metal compared to healthy patients. Once again the interaction between metal type and disease/health status was not significant.

Discussion

At present, the most commonly used material for the use of dental implants is medical grade titanium. Titanium is so widely used due to its many properties that have shown long term clinical success; these include biocompatibility, biochemical and biomechanical properties(143). Despite its many superior qualities, titanium displays less than ideal frictional properties and shear strength, along with a modulus of elasticity dissimilar to bone(143). Titanium implants have been proved to have a reduced capability for osseointegration in systemically compromised patients(144, 145). With millions of Americans affected by some sort of systemic disease and the aging population ever increasing, there is a push for improved biomaterials that have a quicker and more robust osseointegration in healthy as well as medically compromised patients. PTTM appears to be

a viable alternative to traditional titanium implant, providing increased mechanical retention and a microstructure that resembles cancellous bone(125). Tantalum has several advantages over conventional titanium implants, some of which include strength, high porosity, and high coefficient of friction(124). It is these characteristics that have brought it to the forefront of implant dentistry after the results from orthopedic implant studies.

Histological analysis via bone to implant contact both as a percentage and a total was utilized to analyze histologic samples. Currently the gold standard for research based evaluation of implant stability is histologic evaluation of BIC percentage(91). This gold standard was used, however, BIC total was also calculated due to the morphology of the PTTM, which drastically increases the surface area. The increased surface area and the morphology of the PTTM material allows for enhanced clot stabilization and lends itself increased bone to implant contact. In Figure 2.13 the BIC percentage demonstrated titanium test cylinders had increased BIC. This data may over estimate the amount of bone present due to the decreased surface area for the titanium test cylinders, while the PTTM test cylinder surface area was drastically increased.

The timing of the histological sampling may have contributed to the limited amount of bone present in all samples. Previous studies have shown a similar BIC percentage to those found in our study for titanium implants at 4 weeks, however, they demonstrated increased BIC percentage when analyzed at 3 months(146). Previous studies evaluating dental implant healing through the use of human histology retrieved samples at a 6-week time point. Moreover in histologic samples were taken retrieved at 6 weeks in a study evaluating implant healing in type IV bone(147).

Furthermore the three dimensional structure made obtaining a crisp and completely focused image difficult and required the use of imaging software to splice together multiple slices. These multiple slices may have had focus on the PTTM structure rather than the osseous structure, which would have revealed more osseous contact. In addition to previously mentioned factors, small sample size, especially in the disease group may have been a contributing factor for the lack of significance. A larger sample size would also provide more power to detect differences between the groups.

In addition to sample size and timing of histological sampling, the use of oral bisphosphonate therapy may have acted as a confounding factor in histologic samples. Bisphosphonates inhibit osteoclast function, this inhibition favors bone formation and in turn promote increase bone mineral and density(62). Animal models show that systemic treatment with bisphosphonates can increase new bone formation around dental implants(148). A requirement for inclusion in the study was the use of oral bisphosphonates to confirm the diagnosis of osteopenia or osteoporosis, this may have inadvertently created a confounding factor and may explain the trend for osteoporotic patients demonstrating more BIC percentage and total BIC in both titanium and tantalum test cylinders. Although this study was looking at early osseous healing changes in relation to different dental implant materials in healthy and osteoporotic patients, patients taking bisphosphonates tend not to have complications with early osseous healing. Recent research suggests that patients taking bisphosphonates may be at an increased risk of peri-implant bone loss after osseointegration rather than implant loss resulting from osseointegration failure(149). This suggests that the mechanism of bisphosphonates results in impaired bone remodeling and turnover around peri-

implant tissues, resulting in high risk for peri-implant bone loss and potentially late failures(149).

Although not quantitatively analyzed, it was noted during histologic analysis that the distance between the old native bone and the PTTM test cylinder compared the threaded Ti test cylinder was larger. This observation is due to the differences in osteotomy preparations for the test cylinder. The conventional titanium threaded test cylinders could be under prepared and the threads would allow for an intimate fit of the threads to the old native bone. However, the PTTM test cylinder required exact osteotomy preparation due to lack of threads and the press fit nature of this particular test cylinder. This distance of native bone to the implant surface may have been another confounding factor for the trend for BIC % favoring the conventional titanium.

Overall the histologic data showed a no significant differences in comparing the two metals, with the exception of osteoporosis titanium test cylinder mean BIC % to that of healthy trabecular patients. All other comparisons were not significant for mean BIC % and mean total BIC. There was, however, a trend for increase bone formation in osteoporosis/osteopenia patient samples for both titanium and PTTM test cylinders. The total BIC trended in favor of the PTTM, however the BIC % trended in favor of the Ti test cylinders. The BIC % favored Ti due to the decreased surface area and the distance between native bone and the test cylinder surface.

Aside from obtaining an insight in the human histologic analysis around conventional titanium implants and PTTM implants associated with osseous healing around dental implants in health and osteopenia/osteoporotic patients, it was concluded that patients with PTTM implants present important differences in osseous healing compared to animal

studies. It was also determined that the evaluation of PTTM implants must be analyzed differently than conventional implants due to the different surface characteristics as well as distinctive initial bone contact. These findings suggest that the critical events associated with bone formation during the process of osseointegration are influenced by the surface of the implant, and in particular by the cell– implant interface. Compared to Ti, PTTM exhibited a more robust response towards early bone formation and mineralization, which may potentially enhance osseointegration.

CHAPTER 3: CONCLUSION

Our results indicate that patients with osteopenia/osteoporosis demonstrated there were no significant differences in histologic osseous healing at 4 weeks between healthy and osteopenic/osteoporotic patients, and the literature demonstrates controversy as to whether these diseases affect osseous healing. Histologic analysis revealed no statistical significance between the healthy and the osteopenia/osteoporosis group differences of BIC percemtage or total BIC at 4 weeks, however, a difference may be seen at a later time point or after remodeling is completed. Although on initial evaluation no difference was found, much was learned about how to evaluate the two different surfaces/metals in the future. PTTM morphologically has the ability for enhanced clot stability and displayed osteoid structures penetrating within the porous structure. This osseous penetration could allow for increased osseointegration and implant stability. With several other systemic conditions that could affect osseous healing around implants, PTTM demonstrates a potential alternative option for conventional implant therapy due to up-regulation of specific genes involved in osseous healing. The clinical implications of this study are that better understanding of the process of osseointegration can lead to improved treatment strategies by targeting pathways involved early on in the process. It will also allow for the incorporation of PTTM in conventional dental implant therapy and further promote early osseointegration. This can be completed through the use of novel biomaterials or mimicking three dimensional organic macrostructures, leading to improved treatment strategies aimed at enhancing osseointegration and long term success of dental implants in healthy individuals and patients

with osteoporosis. Further research is needed to better evaluate histologic analysis of healing at a later time point with supplementation with molecular data to confirm if PTTM induces a more robust early osseointegration.

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