HISTOMORPHOMETRY AND QUANTATIVE ANALYSIS OF LONG-TERM INPLANT INTERFACES

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Objective: This investigation was to evaluate (>10 year) bone response around custom (HA plasma coated) dental implants and HA particulate. Materials and Methods: Three individual post mortem mandibular Custom Osseous Integrated Implants with surrounding bone were processed at six transverse locations to obtain non-decalcified ground sections 20–80µm thick (n=36) and stained initially with methylene blue/basic fuchsin. Slides were evaluated to select (n= 8) slides for regions of interest along the perimeter of the implant, using optical microscopy (Olympus BX51 microscope) with a Retiga EXi color digital camera (Olympus, Center Valley, PA) and Bioquant VR software (R&M Biometrics, Nashville, TN) at an original magnification from 1-100X and examining 1 mm around implant component. Additional non-decalcified sections were processed (n=5), TRAP stained and examined, focused on osteoclast cells that were identified by TRAP staining. Histomorphometric analyses showed peri-implant area
coverage of residual CaP coating at 46.6% for all specimens. TRAP staining showed the most osteoclastic activity along CaP surfaces, followed by the CaP implant-coating and the residual alloy implant to bone contacting regions. Due to a small sample size statistical analyses were not utilized. Conclusions: Analyses of bone-implant interfaces and osteoclasts from non-decalcified sections of three patients (>10 year) post mortem custom dental implants showed very different interactions where CaP implant-coatings were being replaced, particulate CaP were partially replaced, and osseous integration along all types of implant surfaces was maintained.

Key Words: bone, hydroxyapatite, dental implants, osteoclasts, TRAP staining
DEDICATION

This thesis is dedicated to my parents, who supported me each step of the way.

Also, this thesis is dedicated to my wife and my sons who have been a great source of motivation and inspiration.

Finally, this thesis is dedicated to my sister and my brother in-law who have supported me all the way since the beginning of my studies.
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1. INTRODUCTION

Overall considerations include conditions where oral health is essential to general health and quality of life. It is a state of being free from mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal disease, tooth decay, tooth loss, and other diseases and disorders that limit an individual’s capacity in biting, chewing, smiling, speaking, and psychosocial well-being. Dental caries is one of the world’s most common diseases, affecting approximately 80% of the developed countries population [1]. As per the report by U.S. Department of Health and Human Services, in United States, dental caries is a most common chronic childhood disease, diagnosed at least five times more frequently than asthma [2]. Dental caries is the single most common chronic disease of childhood [3]. However, in recent years, dental caries has declined dramatically among children as a result of various preventive regimens [4]. Since the 1970s, there has been a 57.2% decrease in Decayed, Missing, and Filled Teeth (DMFT) and a 58.8% decrease in Decayed, Missing, and Filled Surfaces (DMFS) in permanent teeth among 6- to 18-year-olds [5,6]. However, despite the reduction, dental caries remains a significant problem in some populations, particularly certain racial and ethnic groups, and among poor children. Pathology from caries is a leading cause of tooth loss.
Another significant impact factor on oral health comes from the second most common oral disease, periodontal disease. According to U.S. Department of Health and Human Services, the prevalence of periodontal disease in adults has decreased from the early 1970s until the latest (1999-2004) National Health and Nutrition Examination Survey. In spite of this improvement, significant disparities remain in some population groups. For the purposes of epidemiological research, periodontal disease is defined very specifically. For a person to have periodontal disease, he or she must have at least one periodontal site with 3 mm or more of attachment loss and 4 mm or more of pocket depth. Moderate periodontal disease is defined as having at least two teeth with interproximal attachment loss of 4 mm or more or at least two teeth with 5 mm or more of pocket depth at interproximal sites. Severe periodontal disease is defined as having at least two teeth with interproximal attachment loss of 6 mm or more and at least one tooth with 5 mm or more of pocket depth at interproximal sites. Prevalence studies showed that 8.52% of adults age 20 to 64 have periodontal disease. Older adults, Black and Hispanic adults, current smokers, and those with lower incomes and less education are more likely to have periodontal disease. Also, the prevalence of severe conditions showed that 5.08% of adults 20 to 64 have moderate or severe periodontal disease. Older adults, Black and Hispanic adults, current smokers, and those with lower incomes and less education are more likely to have moderate/severe periodontal disease. Periodontal disease along with caries is the major cause of tooth loss. According to the National Institute of Dental and Craniofacial Research (NIDCR), tooth loss is a sensitive indicator of overall dental health and access to dental care. Although the overall prevalence of
partial and total tooth loss in seniors has decreased since the early 1970s, significant disparities remain in some population groups.

The National Health and Nutrition Examination Survey (NHANES) is an important source of information on oral health and dental care in the United States. Results of their most recent survey (1999-2004) show that White, non-Hispanic seniors aged 65-74 years have an average of 19.39 remaining teeth. Black seniors of the same age group have fewer remaining teeth (15.19). The NHANES information also breaks down the percentages of senior Americans in different minority groups who have no remaining teeth. Similar disparities exist between the percentages of White, non-Hispanic and Black, non-Hispanic seniors who have no remaining teeth. The percentage of White, non-Hispanic seniors (age 56-74 years) with no remaining teeth is 26.12%. For Black, non-Hispanic seniors of the same age group, the percentage is 32.81%. This study also showed that regardless of age, smokers and those with lower incomes and less education are more likely to have fewer remaining teeth as well as no remaining teeth. This suggests that although the number of Americans with partial and total tooth loss has been declining since the 1970s, it is not declining as rapidly in certain minority groups. Tooth loss negatively affects our quality of life by making eating, chewing, laughing and talking less enjoyable. It also negatively affects our overall health by causing us to substitute high fiber foods for high calorie, high carbohydrate foods that are softer and easier to chew.

The good news is that dental profession has new options for replacing missing teeth. For patients with partial or total tooth loss, dental implants can be used to support new replacement that look, feel, and function like the original teeth. With dental implants,
those with missing teeth can resume a healthy diet, and can laugh, smile, and eat with confidence. Worldwide dental implants are poised to achieve significant growth as patients become aware of the health benefits achieved from having full oral function. Dental implants are in the category of optional surgery and represent the type of consumer spending that is postponed in challenging economic environments. The companies participating in the dental implant market have found ways to manage infection, providing a higher implant success rate. Implantable devices are more stable in the mouth, permitting fewer dental implant failures. 69% of adults ages 35 to 44 have lost at least one permanent tooth to an accident, gum disease, a failed root canal, or tooth decay. By age 74, 26% of adults have lost all of their permanent teeth. With the number of Americans over 55 growing by 60% in the next 20 years there a significant U.S. market opportunity. The baby boomer generation buying power will exceed 3 trillion US dollars in 2013. Implant based treatments has resulted in an ever-increasing number of dental implant systems are appearing on the market. Their design can be loosely categorized as follows: 1. supraosseous or subperiosteal; 2. intraosseous; 3. transosseous (mandibular staple); 4. intramucosal; and intradental (endodontic stabilizers). Although, intraosseous group is the most abundant and well accepted among the dental profession, this paper will further address subperiosteal implant design. Complete subperiosteal implant placement was first described as a treatment for the atrophic mandible in the 1940s. A subperiosteal implant refers to a type of dental implant that is placed in between the periosteum and the alveolar bone. It usually has two to four transmucosal elements (posts) projecting through the mucosa into the oral cavity, connecting the implant to the intraoral prosthesis. Traditionally, subperiosteal implants are made from
cobalt or titanium alloys [7, 8] and often immediately loaded with a removable or fixed overdenture. They are usually placed above the bone and used in cases of severe bone resorption of the ridges [9, 10]. With very advanced jawbone resorption there may not be enough bone width or height for the root or plate form type of implant. In these cases the subperiosteal implant may be prescribed. The subperiosteal implant is custom made and designed to function along the upper surface of the bone, but under the periosteum and oral mucosa. There are two methods for its placement. The "dual surgery" method includes a flap surgery, an impression of the exposed bone and laboratory fabrication of custom framework. A second flap surgery is then carried out for the implant placement. For the "single surgery" method, a CAT scan of the bone is utilized with the CAT scan data and advanced computer modeling techniques to construct a custom framework. A flap surgery is then carried out for the subperiosteal implant placement. While applications of subperiosteal implants are limited today, there are still a small number of patients who survive with stable subperiosteal implants. In some situations, these patients may be presented with an option of implant removal, followed by grafting and placement of endosseous implants prior to the fabrication of a definitive prosthesis [11]. However, most of these patients are elderly and may have general health problems or may simply not want to pursue this option due to the complexity of the treatment and financial concerns. Procedures are often selected due to the high success rates of osseointegrated implants in atrophic mandibles often facilitated by the placement of autogenous grafts. However, as this report highlights, there may still be some options which could be of interest to the dental profession [28]. From a historical perspective, the Maya civilization has been shown to have used the
earliest known examples of endosseous implants, dating back over 1,350 years. During the excavation of Maya burial sites in Honduras in 1931, archaeologists found a fragment of mandible of Maya origin, dating from about 600 AD. This mandible, which is considered to be that of a woman in her twenties, had three tooth-shaped pieces of shell placed into the sockets of three missing lower incisor teeth. For forty years the archaeological world considered that these shells were placed after death in a manner also observed by the ancient Egyptians. Amadeo Bobbio, a Brazilian dental academic, studied the mandibular specimen and took a series of radiographs, back in 1970. He noted compact bone formation around two of the implants which led him to conclude that the implants were placed during life. In the 1950s research was being conducted at Cambridge University in England to study blood flow in vivo. These workers devised a method of constructing a chamber of titanium which was then embedded into the soft tissue of the ears of rabbits. In 1952, a Swedish physician, P I Brånemark, was interested in studying blood flow during bone healing and regeneration, and adopted the Cambridge designed ‘rabbit ear chamber’ for use in the rabbit femur. Following several months of study he attempted to retrieve these expensive chambers from the rabbits and found that he was unable to remove them. Thus, Dr. Brånemark observed that bone had grown into such close proximity with the titanium that it effectively attached to the metal. Dr. Brånemark carried out many further studies into this phenomenon, using both animal and human subjects, which all confirmed this unique property of titanium, when implanted into bone. Dr. Leonard Linkow placed his first dental implant in 1952, four months after he graduated from dental school. By 1992, a dentist Dr. Linkow had placed over 19,000 dental implants and stopped counting. He retired from private practice in 2002 leaving a
body of work that included 12 books and 36 patents. Many implant dentists refer to Dr. Linkow as the father of modern implant dentistry [12]. Meanwhile, a medical doctor from Italy named Stefano Melchiade Tramonte, understood that titanium could be used for dental restorations. After designing a titanium screw to support his own dental prosthesis, Dr. Tramonte started to use it on many patients in his clinic in 1959. The good results of his clinical studies on humans were published in 1966 [13]. Although Brånemark had originally considered that the first work should center on knee and hip surgery, he decided that the mouth was more accessible for continued clinical observations and the high rate of edentulous in the general population offered more subjects for widespread study. He termed the clinically observed bone attachment to titanium as ‘osseointegration’. In 1965 Brånemark, who was by then the Professor of Anatomy at Gothenburg University in Sweden, placed the first titanium dental implant of this design into a human volunteer. Contemporary independent research in the United States by Stevens and Alexander led to a 1969 US patent filing for titanium dental implants [14]. Over the next fourteen years Brånemark published many studies on the use of titanium in dental implantology until in 1978 he entered into a commercial partnership with the Swedish defense company, Bofors AB, for the development and marketing of dental implants. With Bofors (later to become Nobel Industries) as the parent company, Nobelpharma AB (later to be renamed Nobel Biocare) was founded in 1981 to focus on dental implantology. To the present day over 7 million Brånemark System implants have now been placed and hundreds of other companies now produce a wide range of dental implants. The majority of dental implants currently available are shaped like small screws, with either tapered or parallel sides. They can be placed at the same time as a
tooth is removed by engaging with the bone of the socket wall and sometimes also with the bone beyond the tip of the socket. Current evidence suggests that implants placed straight into an extraction socket have comparable success rates to those placed into healed bone [15]. The success rate and radiographic results of immediate restorations of dental implants placed in fresh extraction sockets have been shown to be comparable to those obtained with delayed loading in carefully selected cases [16]. Some current research in dental implantology is focusing on the use of ceramic materials such as zirconia in the manufacture of dental implants. Zirconia is the dioxide of zirconium, a metal close to titanium in the periodic table and with similar biocompatibility properties [17]. Although generally the same shape as titanium implants, zirconia, which has been used successfully for orthopedic surgery for a number of years, has the advantage of being more cosmetically aesthetic for dental applications, owing to its bright tooth-like color [18]. However, long-term clinical data will be necessary before one-piece Zirconia implants can be recommended for routine practice [19].

Dental implant success is related to operator skill, quality and quantity of the bone available at the site, and the patient's oral hygiene and oral health practices. The consensus is that implants demonstrate a success rate of around 75% over the long term [20]. One of the most important factors that determine implant success is the achievement and maintenance of implant stability [21]. The stability is presented as an ISQ (Implant Stability Quotient) value. Other contributing factors to the success of dental implant placement, as with most surgical procedures, include the patient's overall general health and compliance with post-surgical care. Failure of a dental implant is often related to the failure of the implant to osseointegrate correctly with the bone, or vice-versa. A dental
implant is considered to be a failure if it is lost, mobile or shows peri-implant bone loss of greater than 1.0 mm in the first year and greater than 0.2 mm a year after.

Dental implants are not susceptible to dental caries but they can develop peri-implantitis. This is an inflammatory condition of the mucosa and/or bone around the implant which may result in bone loss and eventually implant failure. The condition is usually, but not always, associated with a chronic infection. Peri-implantitis is more likely to occur in heavy smokers, patients with diabetes, patients with poor oral hygiene and cases where the mucosa around the implant is thin [22]. In this regard, currently there is no universal agreement on the best treatment for peri-implantitis. The condition and its causes is still poorly understood [23]. Risk of failure is increased in smokers. For this reason implants are frequently placed only after a patient has stopped smoking as the treatment is very expensive. More rarely, an implant may fail because of poor positioning at the time of surgery, or may be overloaded initially causing an osseointegration failure. If smoking and positioning problems exist, clinicians often do not consider an implant for their treatment planning solution. Failure may also occur independently of the causes outlined above. Implants like any other object suffer from wear and tear. In the majority of cases where an implant fails to integrate with the bone and is rejected by the body the cause is unknown. It has been reported that, this occurs in around 5% of cases. However, we still do not fully understand why bone will integrate with titanium dental implants and why it does not reject the material as a 'foreign body'. There have been a large numbers of theories that tried to explain this phenomenon. A recent theory argues that rather than being an active biological tissue response, the integration of bone with an implant is the lack of a negative tissue response. It has further been postulated that an implant rejection
occurs in patients whose bone tissues actually react as they naturally should with the 'foreign body' and reject the implant in the same manner that would occur with most other implanted materials [24].

There is a consensus, which a high degree of osseointegration would be a key factor for long term implant survival, regardless of the implant type used. Therefore that statement will be further elaborated with a Custom Osseous Integrated Implant (COII) a version of subperiosteal implant system, which was the focus of this investigation.
2. OBJECTIVES

The overall objective of this study was to investigate histological and histomorphometrical properties related to the cellular mechanisms leading to the in vivo stability, partial stability, or instability of functional implant interfaces (until death at about 11 years post-surgery and restoration) for HA (CaP) particulate bone graft, HA (CaP) coating on alloy, and Co-Cr-Mo alloy, implant surfaces. The two specific aims of this study were as follows.

- To investigate the mechanisms of bone interaction with each of the biomaterials, (HA) (CaP) particulate bone graft, HA (CaP) coating on alloy, and Co-Cr-Mo alloy) based on observations focusing on bone remodeling under conditions of previous functional loading.

- To identify the type of cells that exist at sites of bone remodeling and interaction along bone to-alloy; HA coating and HA particulate interfaces.
3. NULL HYPOTHESES

NULL HYPOTHESIS PART ONE

- Bone remodeling of implant/bone, implant HA coating/bone and HA particulates/bone interfaces after 10+ years of functional loading will show a cell mediated mechanism of implant/bone and HA particulate/bone remodeling

NULL HYPOTHESIS PART TWO

- Multinucleated cells associated with bone remodeling mechanism, will consist primarily of osteoclasts
4. LITERATURE REVIEW

Organization of this section is as follows: background information regarding dental implants and the bone-implant interface, followed by a review of the Custom Osseous Integrated Implant (COII), associated bone grafting materials and finally implant to bone integration. Information will include their clinical benefits, advantages and disadvantages as well as challenges that may be encountered by clinicians.

4.1 DENTAL IMPLANTS AS A SOLUTION FOR TOOTH LOSS

The history of modern dentistry is to restore the patient to normal function, speech, health and esthetics, regardless of the atrophy, disease, or injury of the stomatognathic system. Responding to this ultimate goal, dental implants are one option for people in good general oral health who have lost a tooth or teeth due to periodontal disease, an injury, or some other reasons. Implant dentistry is a valid and predictable treatment option for the rehabilitation of partially and completely edentulous arches. An important contribution to this field has been provided by the continuous quality improvement of prosthetic components. At present, a number of implant-supported prosthetic solutions can satisfy patients’ expectations regarding esthetics and function.
The significant advances in dentistry during the twentieth century; unquestionably none has extended the treatment horizons more than the successful use of bone integrated implants. The osseointegration brought new horizons to dental profession. Dr. Bränemark have defined osseointegration as a direct structural and functional connection between ordinary healthy bone and the implant surface, as seen at the level of optical microscopy, producing stability and allowing the structure to support the masticatory forces after one year of functional loading [26].

The custom Osseous Integrated Implant is a custom fabricated cobalt or titanium framework, designed to rest on top of rather than into the mandibular bone and it provides stability through bone support [27, 28]. Permucosal extensions provide support and attachment of the intraoral prosthesis. For stabilizing removable dentures, use of the subperiosteal implant has declined in recent years in favor endosseous dental implants. In some cases, however, the use of endosseous implants may be rejected by patients because of increased cost, time of treatment, postoperative pain, and the introduction of multiple invasive procedures (e.g., hip grafting), i.e., simply to gain stability and to increase function of masticatory function of a lower overdenture.

4.2 BONE IMPLANT INTERFACES

The process of bone remodeling, which involves many cellular steps is not yet fully understood. This interaction represents in essence equilibrium of action–reaction of two major cells at the cellular level: osteoblasts and osteoclasts [29]. Bone change occurs
continuously throughout life to maintain skeletal mass and calcium balance. This involves coupled bone formation and resorption, which are carried out by osteoblasts and osteoclasts respectively. The osteoclast is a multinucleated cell specialized to provide bone resorption. Osteoclasts are not commonly seen at all locations for adult bone but are often found at sites of normal and abnormal remodeling plus osteolysis in diseases affecting bones and joints. Cellular and hormonal/humoral factors which influence the extent of bone resorption act by regulating the activity and number (i.e. formation and survival) of osteoclasts. From a dental prospective, the loss of teeth results in resorption of the alveolar process and, in more advanced stages, resorption of the underlying basal bone. A severely resorbed mandible generally results in problems for the lower intraoral prosthesis, such as insufficient retention, pain by overloading the mucosa, impaired masticatory function, speech difficulties, loss of soft tissue support, altered facial appearance, and psychosocial problems.

The bone-implant interface develops according to the host tissue response to the implant surface, which can be bioinert, biotolerant, or bioactive, depending on the implant surface, chemical composition and topography [30-32]. However, roughening of the topography of the implant surface by applying a porous coating or through surface treatments may promote osteogenesis by enhancing osteoblast metabolic activity and cellular adhesion. Also there may be an increased available surface area, thus helping to stabilize the fibrin scaffold, with the ultimate goal of increasing bone integration. At the histologic level, faster bone apposition may be achieved with roughened surfaces compared to machined surfaces [33]. In one study, a nearly linear relationship was found between tensile failure load and surface roughness with higher removal torques for
hydroxyapatite (HA) coated implants in comparison to other implants [34-36]. Plasma-sprayed hydroxyapatite (HA) coatings have been used as surface coatings on metallic implants in dentistry and orthopedics since the mid-1970s. The advantages that are sought in this application include more rapid and stronger fixation between the host bone and the implant, plus increased and more uniform bone ingrowth and/or ongrowth at the bone-implant interface [37]. Although little clinical advantage was found in some trials with HA-coated implants, most clinical experience with either weight-bearing or non-weight bearing models have shown promising results shortly after the implantation with continued fixation for up to 10 years [38]. Furthermore, HA coating can enhance bone growth across a gap of 1 mm between the bone and the implant for both stable and unstable mechanical conditions thus limiting the formation of any fibrous membrane and converting a motion-induced fibrous membrane into a bony anchorage [39]. In HA-coated implants, one of the events occurring at the bone-implant interface is the resorption of the HA coating, also called degradation or coating loss, sometimes with the presence of HA bone grafting particles. Although it is essential for the establishment of bone-implant integration, this has been one of the main concerns for the durability of the HA-coated implants. Partial dissolution of the HA coating may be essential to trigger bone growth, plus some crystalline coatings have been proposed to promote earlier bone growth and stronger fixation. Overall, bone remodeling proceeds over time that is, osteoclasts resorb normal bone by actively secreting hydrogen ions into the extracellular space, creating a local pH of approximately 4.8. Although the HA coating may be very stable at neutral pH, they are more soluble at these acidic local environments, and the low-crystallinity coating shows even more rapid dissolution. Thus both the low-
crystalline carbonated HA in bone mineral and the HA lower density coating can be removed at pH 4.8. This resorption of the coating as part of normal remodeling which is proposed as the main coating loss mechanism in the long run as has been shown in many time-related studies using histological specimens [40]. The coating can be removed with time as a response to this bone remodeling process, where the removal of coating was usually replaced with the new bone, especially in some areas with load transfer, suggesting the acceleration of coating resorption and bone remodeling with functional loading [41-43].

4.3 CUSTOM OSSEOUS INTEGRATED IMPLANT (COII)

Reconstruction of a severely resorbed mandible to restore oral function remains a surgical and prosthetic challenge due to the minimal amount of residual bone support and the progressive nature of the resorptive process [44]. In those kind of situations, treatment options would not include an intraosseous (endosseous) implant supported prosthesis. Vertical augmentation of the alveolar ridge is necessary for patients with extensive resorption of the alveolar ridge in order to often perform aesthetic and prosthetic rehabilitation and enable Custom Osseous Integrated Implant insertion. Dr. Douglas Martin began using Custom Osseous Integrated Implants (COII) in 1985 using techniques expanded by Dr. Tom Golec. As an extension, Dr. Martin began post mortem harvesting atrophic mandibles previously implanted system to better understand viability of the system over the life of a patient. The mandibles that he retrieved, when sectioned
and analyzed, showed osseous integration of Vitallium® ASTM F-75 alloy. His pioneered work with COII was a novel approach at that time. Dr. Martin has been involved in research on Custom Osseous Integrated Implants with the University of Alabama at Birmingham since 1995. (IRB# X050823001)

Animal and human retrievals of previous design of metallic surfaced subperiosteal implants showed that the implant's interface to the underlying bone is compressed fibrous tissue [45]. For example, Bodine's 15-year follow-up report of a mandibular subperiosteal implant indicated that epithelial migration was limited to 3 mm from the perimucosal post region. From these observations and others, such as James, the metallic surfaced subperiosteal implants developed a region of soft tissue at the implant to bone interface [45, 46]. This fibrous tissue interface has been implicated as the reason for a long-term subperiosteal implant survival of only 75% or less after 10 years. Compressed fibrous tissue, devoid of inflammatory cells, enveloped the metallic implant [46]. During the same period, hydroxyapatite-(HA) coated titanium and cobalt alloy implants were used as an endosseous dental implant material combination. When placed in bone, the HA coating interacts with bone without a demonstrable soft-tissue seam [34]. For many applications, when placed under periosteum and over intact cortical mandibular bone, dense HA blocks also form a direct connection with the underlying cortical bone without a detectable soft-tissue seam. These observations form the basis for redesigning and the coating the prior subperiosteal design implants with HA.
4.4 BONE GRAFTING MATERIALS

The fixed prosthetic replacement of lost human teeth is dependent on the presence of reliable abutment teeth. The prognosis of these teeth can be compromised by pathologies such as cystic defects, periodontal pockets and surgical bone defects. Furthermore, the collapse of the residual alveolar ridge after tooth extraction or surgery can jeopardize the aesthetic result of fixed partial dentures. The retention and stability of removable dentures is primarily dependent on the size and shape of the residual alveolar ridge [47]. This stability and retention of complete dentures will be compromised by the continuous resorption of the alveolar ridge, so that it will become impossible for the patient to wear and function with dentures. Reconstruction of osseous deficits is often provided with calcium phosphate ceramic particulates. These materials act as filler biomaterials, and new bone tissue formation takes place along their surface. Dense and porous hydroxyapatite (HA), as well as tricalcium-phosphate (TCP) particulates have been used as such filler materials. These materials are also used for the augmentation of resorbed alveolar ridges. The rational for use of these particulates is to provide a scaffold for enhanced bone tissue repair, growth and stability. Both experimental animal models and clinical reports have provided evidence of bone growth along these particulate materials, which act as a scaffold. However, such observations have not been seen consistently. Particles near the wall of the lesions have been incorporated in the bone regrowth, but in the center of the lesion or in extra-osseous sites some particles are encapsulated by fibrous tissue. This resulted in a lack of consolidation of the particles, their migration from the surgical sites and even the exfoliation of the particles [48].
There have been a small number of studies that focused on improving the ease of surgical manipulation and the subsequent consolidation of the particles [47]. As an example, the invagination of the epithelial cells in periodontal pockets is not prevented by the use of these type biomaterials, regardless of their osteoconduction. Treatments often resulted in formation of a long epithelial junction, although the remaining teeth appeared to function very well clinically [49].

Bioactive glass is another bioactive ceramic-like that can be used as a particulate. It has the ability to bond to bone tissue and, in addition, it enhances bone tissue growth due to its osteoconductive properties [50-52]. The bonding mechanism between these glasses and the surrounding bone tissue appears to be the result of a series of interfacial reactions that lead to the formation of a Si-rich layer covered by a CaP-rich layer. It has been suggested that osteoblasts deposit the organic matrix of bone on this CaP-rich layer, and that the bonding results from cross-linking between ionic sites on the collagen and the mucopolysaccharides, with sites on the CaP-rich layer [52]. In addition to that, the continuous growth of the CaP-rich layer leads to a CaP-organic component structure, which leads to formation of a continuous structure [50]. Since bioactive glasses have been shown the aspect of the soft tissue integration in various situations, it is conceivable that their encapsulation in fibrous tissue will not lead to migration, as occurs with the CaP ceramics [52]. In a comparative study with two hydroxyapatite (HA) materials (Calcitite® and Interpore- 200®), bioactive glass granules showed good or even an enhanced tissue response, when compared to the other biomaterials [52]. The three materials investigated in this study had a positive effect on bone repair. It has been shown that bone growth started from the cavity wall at the cortical bone border, using the
granules as a scaffold for osteoconduction. This process occurred massively around the bioactive glass particles, as many particles were entirely embedded by bone tissue, resulting in a very dense bone structure. On the other hand, in the center of the cavity just below the cortical bone border, trabecular bone was adherent to the glass particles, giving this area a plate-like nature. Fibrous tissue was present between these trabeculae of bioactive glass particles and trabecular bone. That resulted in a stable structure, which prevented the collapse of the cortex of the original cavities. The repair could be considered sufficiently satisfactory after 3 months of implantation to allow for further prosthodontics treatment. The other two materials showed less osteoconductive bone growth from the cavity wall, fewer particles being surrounded by bone tissue, thus the repaired tissue was less dense. The bone trabeculae were also present in the center of the cavities, which were not often seen in direct contact with the hydroxyapatite granules. These bone trabeculae were separated from the granules by a thin layer of fibrous tissue. Bioactive glass particles compared to the other bioactive materials showed an ability to stimulate the formation of new bone tissue. It has been clearly demonstrated that most glass particles are eroded internally via small cracks. These small cracks appeared to be excavated centers, the sites where new bone formation was detected and they were not connected to external bone tissue [51, 52].
Since the first introduction of the term “osseointegration” by Bränemark in 1969, the use of the word osseointegration has become associated with a highly viable and predictable treatment modality in modern implant dentistry [26]. However, many unanswered questions remain regarding in-vivo osseointegration mechanisms that regulate the bone response to the surface property variables at micro to nano-scales. It is widely believed that osseointegration exists in part through biomechanical interlocking or inter-digitation that is established as bone grows into and onto surface irregularities over time. This interface becomes stabilized by dynamic regulation of bone modeling/remodeling mechanisms. The mechanisms by which endosseous implants become integrated in bone can be subdivided into three separate phenomena, each of which have been tested experimentally. These three biologic phenomena are elaborated in more details below, since they have a tremendous influence on design, treatment planning and overall long-term implant longevity. Therefore, there are many fundamental questions to be answered, related to the mechanisms by which bone may be formed on an implant surface, and the progress of bone growth towards and along an implant surface. This distinction was explored by Osborn and Newesley, who described the two different phenomena of distance and contact osteogenesis [53]. The bone-implant juxtaposition that occurs was based on a sound understanding of the recruitment of osteogenic cells to the implant surface and their anabolic behavior and also on an acceptance of the multitude of tissue-implant interfaces that are created, even in a bony compartment, upon implantation of an implant. The terms distance and contact osteogenesis were first
described by Osborn and Newesley in 1980, when they referred to the general relationship between forming bone and the surface of an implanted material [53]. Therefore, in distance osteogenesis, new bone is formed on the surfaces of bone in the peri-implant site. Similar to normal appositional bone growth, the existent bone surfaces provide a population of osteogenic cells that lay down new matrix, which, as osteogenesis continues, encroaches on the implant itself. Thus, the new bone is not forming on the implant itself, but rather that the implant becomes surrounded by bone. It was proposed, in these circumstances, that the implant surface will always be partially obscured from bone by intervening cells and general connective tissue extracellular matrix. Therefore, it was predicted, in such a case, that it would be impossible to achieve the phenomenon of bone-to implant bonding.

In the second description, contact osteogenesis, new bone forms first on implant surface. Since, a priori, no bone was present on implant surfaces upon implantation, the implant surface becomes colonized by a population of osteogenic cells before initiation of bone matrix formation. This could occur also at remodeling sites where an old bone surface or an implant surface is populated with osteogenic cells before new bone is deposited. The common factor in these cases is that bone forms at these sites. The term de novo bone formation to describe such an event and to distinguish this phenomenon from appositional bone growth, where the continuum of bone formation represents the transient anabolic behavior of already differentiated osteoblasts. Clearly, an essential prerequisite of de novo bone formation is the recruitment of osteogenic cells to the site of future matrix formation. While both distance and contact osteogenesis will result in the juxtaposition of bone along the implant surface, the biologic significance of these
different healing reactions is of considerable importance in both attempting to unravel the role of implant design in endosseous integration, and in elucidating the differences in structure and composition of the bone-implant interface. It can be assumed that any endosseous healing compartment will display both distance and contact osteogenesis phenomena. Furthermore, there is no clear difference between healing responses of osteogenic cell migration (osteocoonduction) and de novo bone formation. Subsequently, another tissue response, the bone remodeling, also at discrete sites, may create de novo bone formation at an implant surface [53]. Contact osteogenesis also relies on the migration of differentiating osteogenic cells to the implant surface. Typically, differentiating osteogenic cells are derived, at bone remodeling sites, from undifferentiated peri-vascular connective tissue cellular elements. A more complex environment characterizes the peri-implant healing site since this will be occupied, transiently, by blood. In that case, as in fracture healing mechanisms, migration of the connective tissue cellular elements will occur utilizing the fibrin that forms during blood clot related phenomena. This connective tissue stromal cell population will provide both the early connective tissue that replaces the fibrin and a source of osteogenic cells. It is important that once differentiating osteogenic cells start secreting bone matrix, they stop migrating. Therefore, bone ingrowth into three-dimensionally complex surfaces, will be the result of osteogenic cell migration. Thus, implant surface topography and wetting ability have an important function in fibrin attachment retention during this healing phase which includes wound contraction [53]. During the phase of fibrin retraction, as a result of cell migration, retraction could cause detachment of the fibrin from the implant surface. On the other hand, if the implant surface provides sufficient anchorage of the
fibrin to withstand detachment during cell migration, the region could maintain a migratory pathway for the differentiating osteogenic cells to reach the implant surface. Thus, the cells that differentiate before reaching the implant surface could synthesize bone matrix that would not be in contact with the implant surface. However, other cells will reach the implant surface before attaining the stage of differentiation at which matrix secretion is initiated. Therefore, these cells will then be available to synthesize de novo bone on the implant surface as cells stop migrating. However, other cells, still in migratory mode, will contact the contiguous implant surface and secrete bone [53]. Thus, the phenomenon of osteoconduction relies on the migration of differentiating osteogenic cells to the implant surface. It is also known that the chemistry of some implant surfaces increase both the adsorption and retention of macromolecular species from the biologic milieu, and thus potentiate osteoconduction. This could be an explanation for the overwhelming evidence of accelerated early bone healing around calcium phosphate-based implant biomaterials [54, 55].

The phenomenon of de novo bone formation is a four-stage process that has been described and confirmed by experiments both at implant surfaces and natural bone remodeling sites [56-58]. The theory of de novo bone formation was proposed almost a century ago, explanation using in vitro methods for the formation of this matrix was described including differentiating osteogenic cells in culture in 1991 [59,60]. Differentiating osteogenic cells initially secrete a collagen-free organic matrix that provides nucleation sites for calcium phosphate mineralization. The two non-collagenous bone proteins, osteopontin and bone sialoprotein, were shown to play a major role in this initial organic phase. Also, the substratum does not necessary act as an epitactic nucleator
in this biologic mineralization phenomenon. Calcium phosphate crystal growth follows
nucleation, and concomitant with crystal growth at the developing interface, there will be
initiation of collagen fiber assembly. Finally, calcification of the collagen compartment
will occur, both in associations with individual collagen fibers or in the interfiber
compartment. Thus, in this process of de novo bone formation, the collagen compartment
of bone will be separated from the underlying substratum by a collagen-free calcified
tissue layer containing non-collagenous bone proteins. This layer has been shown to be
approximately 0.5 pm thick, as were cement lines that form the interface between old and
new bone at reversal sites. Such bone matrix formation is dependent on the migration of
osteogenic cells to the implant surface and differentiation to active secretory cells.
The mechanism for the bone-bonding phenomenon is generally accepted to include a
chemical interaction that results in collagen from the bony compartment inter-digitation
with the chemically active surface of the implant. Clearly, in the case of de novo bone
formation, this mechanism is not probable, since the first extracellular matrix elaborated
by bone cells at the implant surface is collagen-free. If bone cement lines exist on both
non and integrating biomaterials, then a reevaluation of the phenomenon of bone
attachment is essential. The degree to which the cement line matrix can be visualized on
bone-bonding materials has been shown to be a function of their chemical surface
reactivity [61, 62]. Even through, chemical hypotheses to explain bone bonding have
been generally adopted in the literature and experimental evidence demonstrating the de
novo bone formation at the implant surface exists, integration is achieved by
micromechanical inter-digitation of the cement line with the biomaterial surface.
Bone remodeling is of particularly critical importance in the long-term stability of the transcortical portion of an endosseous implant, since cortical bone will undergo some necrosis as a result of the surgical trauma to the tissue. During this long-term phase of peri-implant healing, remodeling at osteons that impinge on the implant surface results in de novo bone formation will occur at these specific sites on a transcortical implant. The remainder of the transcortical portion of the implant will be occupied by old, necrotic bone or connective tissue space created by surgery related peri-implant necrosis and lysis of bone tissue. Even through trabecular remodeling also take place in this process, this is only a component to implant stability. As previously mentioned, calcium phosphates may be employed to accelerate early peri-implant bone healing by potentiating osteoconduction through structural protein adsorption and retention during early healing. All calcium phosphate coatings so far reported in the literature exhibit a complex surface topography that would facilitate bone integration. Furthermore, there is now emerging evidence that calcium phosphate coatings can be site-specifically resorbed by osteoclasts. Therefore, it would be expected that new biologic design strategies for dental implants featuring not only osteoconductive and bone-integrative properties, but would also have surfaces that would be easily replaced by bone during normal tissue remodeling.
5. DEVICE RETRIVAL AND ANALYSIS

Mandibular Implants were recovered from three female patients post mortem, ages 74–94 years, after being in vivo for about 11 years each. The implants were made with ASTM F-75®, a non-magnetic Cobalt Chromium Molybdenum (CoCrMo) alloy exhibiting high strength, corrosion resistance, and excellent wear resistance. The alloy was plasma sprayed with HA coating (CaP ASTM-1609). During surgery, vertical augmentation of the mandible with HA particulates (ASTMF-1875) was performed around the Co alloy implants in order to treat a severe bone loss. All three implants were functioning as intended at the time of patient deaths. Laboratory analyses were conducted under IRB approval and in compliance with HIPAA requirements [IRB# X050823001]. The design of the implant included a major connector (with four O rings overdenture attachments) which interconnected anterior (buccal and lingual) with two posterior buccal custom osseous integrated implant struts. Clinical experience has shown that 4 implant post device as being the ideal configuration for distributing the physical and biting forces across the entire jaw. All implant body parts were plasma sprayed with HA coating as shown in (Fig. 1)
When these patients passed away, they and their families donated a section of mandible with implants on it for the research purpose. Radiographic image of severely atrophic mandible with custom osseous integrated implant in place, showing a metal framework spanning the entire edentulous mandible. Also, two dark shaded areas represent the approximate location where the implant was sectioned, as shown below (Fig. 2)
Figure 2. Radiograph of mandibular COI I device and two dark areas represent the sites where sectioning took place.

The implant-mandible constructs were sectioned at the anterior and posterior posts for a total of six sections per mandible. Specimen preparation included controlled dehydration, embedding in methyl methacrylate, sequential polymerizing, grinding under constant water irrigation with silicon carbide paper (320, 800, 1000, and 2500) grit sizes followed by polishing with diamond pastes (3, 0.25 lm) and alumina slurry (0.05 lm). All processed nondecalcified thin sections were stained with methylene blue (Allied Chemicals, NY)/basic fuschin (Sigma-Aldrich, MO) and sonicated with deionized water between grinding and polishing steps and prior to optical microscopy examination. The non-calcified ground section of the mandibular bone with implant and HA particulates at low original magnification at optical microscopy are shown in Figure 3. Please note that buccal strut and alloy framework are shown in black due to lack of transparency. Also, mandibular bone exhibits some discontinuation, possibly due to processing and biomechanical remodeling, non-decalcified of mandibular canal dehiscence. Furthermore, under the implant-bone interface there are large conglomerates of HA particulates (red arrows) surrounded by a large amount of trabecular bone, with lack of bone matrix in the
center of the ground section. The presence of relatively immature bone adjacent to the implants, which was less dense (lower mineralization), indicated by staining characteristics than the interstitial bone distant from the implant is shown (Fig. 3). It is not possible to discern the quality of HA coating present at this low magnification. However, the presence of HA coating can be seen on the tip of the buccal strut.

Figure 3. This image shows a cross section of mandibular bone with COII from patient 1 (black color) shows interface between bone and HA particulate under the implant surface (light gray) with bone (purple).
6. MATERIALS AND METHODS

This study was divided into the following phases: histological and histomorphometrical analysis, TRAP staining and histological and histomorphometrical analysis of TRAP stained ground section. The following section provides a description of the procedures that were employed in this study.

6.1 HISTOMORPHOMETRICAL ANALYSIS

Slides (20-80µm thick, n=36) were examined by optical microscopy using an Keyence/VHX-600 microscope and BioquantVR software (R&M Biometrics, Nashville, TN) at an original magnification of 100. Eight sections with a region of interest along the perimeter of the implant were selected based on cellular activity. Histomorphometric analysis (n=8) was performed by digital optical microscopy (Keyence/VHX-600) 1-1000X magnification, cell count measured by using Bioquant Image Analysis software (Rtm Biometrics/Nashville, TN), and examining 1 mm around implant component circumferences. The final analysis provided the ratio of the number of multinucleated cells per millimeter of bone surface. In order to further investigate type of cell mediated mechanism of bone/coating and HA particulates remodeling, new non-calcified ground
sections (20-80µm) were produced. Specimens (n=5) preparation was conducted utilizing the same protocol as previous specimens preparation.

6.2 TARTRATE-RESISTANT ACID PHOSPHATASE (TRAP) STAINING

Tartrate-resistant acid phosphatase (TRAP), which is a metallophosphoesterase participating in osteoclast-mediated bone turnover, was utilized to identify osteoclasts. In order to enzymatically identify osteoclast cells, the following protocol was used for TRAP staining, which included 0.2M Acetate buffer and 50 mM tartaric buffer solutions. Five freshly cut bone ground sections of 20–80µm thickness, were placed in 0.1 M tris-1mM EDTA buffer pH 9 overnight in 37°C oven. The next day the sections were washed out in deionized water. The slides were placed into the 0.2M Acetate buffer at ambient temperature for 40min and then to same solution 0.5mg/ml naphtol AS-MX phosphate and 1.1mg/ml Fast Red TR (1,5-Naphthalenedisulfonate) salt were added. The slides were incubated in that solution at 37°C for 60 min and washed out thoroughly with deionized water. At this stage, ground sections were ready for optical microscopy. A 10µm thick paraffin section of mice bone was used as a positive control. (Fig. 4)
Fig. 4 Image of paraffin section of mice bone (positive control) with bright red area abundant with TRAP+ osteoclasts cells.

6.3 TRAP HISTOMORPHOMETRICAL ANALYSIS

The TRAP stained slides were examined by optical microscopy using an Olympus BX51 microscope with a Retiga EXi color digital camera (Olympus, Center Valley, PA) and Bioquant VR software (R&M Biometrics, Nashville, TN) at an original magnification of 10X. The histomorphometric parameters included measure of HA coating magnitude and osteoclast count 1mm around implant component circumference, plus the osteoclast
count 1mm around HA particulate component circumferences. Bright red that appeared on TRAP sections represented the enzymatic marker for osteoclasts. In order to further investigate the identification of osteoclasts in the sections, a blue-fluorescent DAPI nucleic acid staining of DNA content and nuclei for cellular imaging techniques was utilized. This method is used as component of high-content screening tests requiring cell-based quantitation of DNA content. All samples including a positive control sample were placed into bath with 1:1000 DAPI solution for 5min (avoiding the light) followed by PBS wash. Then the cover slip was protecting the slides prior to fluorescent light optical microscopy (Olympus BX51 microscope with a Retiga EXi ) and color digital camera (Olympus, Center Valley, PA) at an original magnification of 10X. The purpose of DAPI staining was to see the nuclear stain of Osteoclasts. The image of DAPI on TRAP section of HA particulate (black hollow) is shown in Figure 5. However, due to overlapping nuclei, it was not possible to acquire exact number of osteoclast nuclei, thus the process proved to be confounded and was not utilized for the remaining ground sections. After consultation on statistical analyses, it was decided that analysis of significant numbers would not be possible (low power).
Fig. 5 Image showing DAPI counterstaining on TRAP HA particulate and seen under fluorescent microscopy at 20X original magnification.
7. RESULTS

Histological examination demonstrated that, overall, there was considerable bone-implant integration with apparently no signs of pathology in the analyzed regions for any of the specimens. In some areas the HA coating was removed and the implant surface was covered with a compact-like bone, while other areas showed contiguous bone-HA coating. However, in some samples, the HA coating was lost during the processing phase and these areas were not included in the analysis. A significant cellular activity was observed around the implants circumferences and TRAP staining, while histomorphometric analysis confirmed regional osteoclastic activity. The amount of HA coating was determined to be 46.73±72.9% for specimens from patient 1. However, the amount of coating was 48.72% for a specimen from patient 2 and 47.12% for a specimen from patient 3 respectfully. Thus, overall average remaining HA coating on all implant surfaces was 47.31±73.1%. Interestingly enough, results of HA particulates showed 79.09% of total HA surfaces were surrounded by bone with osteoclasts. Based on the results, HA particulates demonstrated most favorable trends of osteoclast cellular activity confirmed by TRAP staining. Histomorphometric TRAP results for all three patients combined are shown for implants in Fig 6. The results for TRAP treated HA particulates are shown in Figure 7.
Figure 6. Histomorphometric results of Implant Surfaces with Osteoclast cellular activity confirmed by TRAP for all three patients in mm (Mean ± SD) (pooled data). The overall percentage of dissolved HA coating is 52.79%, whereas osteoclasts activity twice as much on HA coating versus non coating implant surfaces.
Figure 7. Histomorphometric results of HA Particulates Surfaces with Osteoclast cellular activity confirmed by TRAP for all three patients in mm (Mean ± SD) (pooled data). The overall percentage of HA Particulates Surfaces with osteoclast activity was 79.09% confirmed by TRAP staining.

### TABLE I. Overall measurements of TRAP Staining for Each Patient (in mm)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total Length of Implant Surface</th>
<th>Implant Coating W/Bone OC+</th>
<th>Implant Coating W/Bone OC-</th>
<th>Implant No Coating W/Bone OC+</th>
<th>Implant No Coating W/Bone OC-</th>
<th>Total Perimeter of HA Particulates Surface W/Bone OC+</th>
<th>HA Particulates Surface W/Bone OC-</th>
<th>HA Particulates Surface Without Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.11 ± 21.1</td>
<td>15.71 ± 12.6</td>
<td>1.67 ± 2.1</td>
<td>0.43 ± 0.7</td>
<td>3.26 ± 3.4</td>
<td>4.55 ± 6.3</td>
<td>12.49 ± 14.7</td>
<td>71.7 ± 11.1</td>
</tr>
<tr>
<td>2</td>
<td>44.03</td>
<td>19.67 ± 12.6</td>
<td>1.78 ± 2.1</td>
<td>0</td>
<td>14.77 ± 12.9</td>
<td>7.89 ± 12.9</td>
<td>49.6 ± 12.9</td>
<td>45.76 ± 12.9</td>
</tr>
<tr>
<td>3</td>
<td>25.19 ± 6.84</td>
<td>14.73 ± 10.1</td>
<td>2.36 ± 10.8</td>
<td>0.26 ± 12.9</td>
<td>7.16 ± 12.9</td>
<td>9.47 ± 12.9</td>
<td>2.73 ± 12.9</td>
<td>53.7 ± 12.9</td>
</tr>
<tr>
<td>pooled</td>
<td>36.67 ± 10.4</td>
<td>14.73 ± 10.1</td>
<td>2.36 ± 10.8</td>
<td>0.26 ± 12.9</td>
<td>7.16 ± 12.9</td>
<td>9.47 ± 12.9</td>
<td>2.73 ± 12.9</td>
<td>53.7 ± 12.9</td>
</tr>
</tbody>
</table>

“Patient 1” includes all specimens sectioned from patient 1 (Mean ± SD); “Patient 2” includes all specimens sectioned from patient 2; and “Patient 3” includes all specimens sectioned from patient 3. Pooled data from all patients (Mean ± SD)
The present study provided novel data regarding properties of bone surrounding HA-coated implants after about 11 years of function and new insight into the role of HA coatings on the nature of the bone-coating interface longer term. According to data acquired, bone remodeling of implant/bone, implant HA coating/bone and HA particulates/bone interfaces after 11 years of functional loading had shown a cell mediated mechanism of implant/bone and HA particulate/bone remodeling. The examples of non-calcified ground section from patient 1 is shown in Figure 8. The light microscopy images showed a partially discontinuous HA coating (light brown). Various degrees of the lamellar compaction of the interfacial bone were present. There is a HA particulate (silver gray) present at the upper left corner (Fig. 8), which is partially surrounded by osteoclasts with irregular pattern along the upper border, indicative of high magnitude cellular activities.
Figure 8. Patient 1: Image from ground section of mandibular bone matrix with the implant (black). Along the implant perimeter, a partially discontinued HA coating and complete implant to bone interface surrounded by lacunae of osteoclasts depicted in bright red. Also HA particulates at left upper corner (silver gray) partially surrounded by osteoclasts.

A ground section of mandibular bone with implant (black) with various degree of HA coating and a few areas of osteoclast activities depicted in red is shown in Figure 9. The densely mineralized collagen fiber matrix of the bone (brown) appears to be spreading parallel to implant surface. On one part of the bone matrix, there was an area with HA particulate surrounded by a highly active region of osteoclast interaction, depicted by bright red and pointed by an arrow. There was a large white area represents just lacking of bone elements, most probably due to processing.
Figure 9. Patient 1: Image of ground section of the bone at low magnification. Implant bone interface on the left (black) has some area of osteoclast activity (depicted in red). The section of the bone (depicted in brown) shows a small area of HA particulates within the bone matrix, surrounded by osteoclasts (white arrow pointing to bright red).

At the higher magnification of the previous Figure 9, shown in Figure 10, mandibular ground section of the bone depicts densely mineralized collagen fiber matrix of the bone (brown) with a single HA region, surrounded by osteoclasts depicted in bright red. Note that remaining surface of HA is significantly resorbed and completely surrounded by an active osteoclast cellular activity. It appears that regional osteoblast are not localized to the HA particulate surface only, but are dispersed around the HA particulate.
Figure 10. Patient 1: Image of ground section of the bone at high magnification higher magnification of the previous figure, showing ground section of the bone matrix with a single HA particulate, surrounded by lacunae of osteoclasts depicted in bright red.
8. DISCUSSION

Based on overall results in this study, the implant-bone and coating-bone interfaces of relatively long-term functional hydroxyapatite-coated Co alloy dental implants of one design (COII), obtained post mortem were characterized. The objective of this study was to investigate the mechanisms of bone interaction with each of the biomaterials, (HA)(CaP) particulate bone graft, HA (CaP) coating on alloy, and Co-Cr-Mo alloy. The study was based on observations focusing on bone remodeling under conditions of previous functional loading. Also, a specific aim was to identify the type of cells at site of bone remodeling and interaction among Implant/Bone and HA particulate/Bone interfaces. The literature supports that bone mass is controlled by a balance between the activity of osteoblasts and the activity of osteoclasts [64]. Alkaline phosphatase and tartrate-resistant acid phosphatase (TRAP) were used as markers for osteoblasts and osteoclasts, respectively. Osteoclasts are the only bone-resorbing cells, under conditions of normal function [65]. Considering the expression of TRAP, which is regarded as an osteoclast marker, although there are other cells of the macrophage/dendritic cell family that express TRAP under certain conditions TRAP, is mostly characterized by its biochemical property, i.e., through its acid phosphatase function, which cannot be blocked by tartrate. Alkaline phosphatase and tartrate-resistant acid phosphatase are used as markers for osteoblasts and osteoclasts, respectively. The histochemical TRAP staining results in a colored stain (red) that can be easily detected using light microscopy. In terms of reliable methods for doing immunohistochemistry
(IHC) on non-calcified sections of bone, there was a common problem with DAPI staining. As it is generally well known, bone is very auto-fluorescent, so most dyes that excite in the visible range also excite substances present in bone itself, making it difficult to find the specific signal generated by the dye-conjugated antibody. However, using DAPI, an intercalating DNA stain used to light up the nuclei, which the background is almost totally black, meaning that bone isn’t auto-fluorescent in the UV range, where DAPI excited. However, that was not a major problem; this was simply corrected with usage of the Alexa594 filter set and higher illumination. Another problem that was impossible to correct was the condition that the bone sections resulted in an overlap of cellular compartments, limiting analyses of DAPI stained nuclei. In addition, bone marrow of the samples had a distinctive spongy appearance in these sections, which could have been from the shrinkage caused by dehydration during processing and storage, from extraction of the fatty deposits by the hydrocarbon solvents used to dehydrate the tissue, possibly from the time elapsed from original acrylic fixation. 

Nerveless, osteoclasts and macrophages are essentially identical in terms of surface epitopes and enzymatic activities, therefore both type of cells would render TRAP+ cells. However, there were two options to deal with this issue. It can be consider only TRAP+ cells on the surface of bone to be osteoclasts, or to perform staining for cathepsin K, which shouldn’t stain macrophages. Under normal circumstances, TRAP is highly expressed by osteoclasts [65], therefore bone surface TRAP+ cells clearly demonstrated osteoclast activity, but didn’t lend itself to quantitative analysis [67]. To further analyze the cell type, consultation with a pathologist provided cell type identification by morphology and characteristics of staining.
A prerequisite for any dental implant is permanent fixation to the surrounding environment with no intervening soft tissue. A successful fixation should be fast and strong initially and exhibit lifelong stability. Fixation takes place by osseointegration, which was first described by Brånemark as the intimate contact between a titanium implant surface and the surrounding bone for a functioning implant system [26]. The currently accepted definition for osseointegration is “contact established between normal and remodeled bone and an implant surface without the interposition of non-bone or connective tissue, at the resolution limits of optical light microscopy. In this study, implant-bone contact was established by histomorphometric analysis of the interfaces. The results were consistent with the presence of relatively immature bone adjacent to the implants, apparently with no any signs of inflammatory reaction on samples used in this study. The histomorphometric analyses showed a mean as 47.31% HA coating of the total implant surface covered by HA coating after roughly 11 years in vivo. The results are in line with reported studies of different coatings at shorter in vivo periods [68, 69]. According to literature, HA dissolution process has been suggested to be initiated with the dissolution of the coating soon after the implantation. This can be described as follows: (a) partial dissolution of HA coating where calcium and phosphate ions are released from the coating, which will cause a rise of the calcium and phosphate ion concentration in the local environment around the coating; (b) precipitation of crystals on HA coating and ion exchange with surrounding tissues; (c) formation of a carbonated calcium phosphate layer of microcrystals and macrocrystals with the incorporation of a collagenous matrix and bone growth toward the implant; (d) bone remodeling in area of stress transfer, where osteoclasts resorb normal bone by actively secreting hydrogen ions.
into the extracellular space, creating a local acid pH and leading to fast resorption of both carbonated HA in bone mineral and the HA coating; and (e) the bone-implant interface was subjected to further bone ingrowth and remodeling, and a biological fixation was achieved through the bidirectional growth of an integrating layer [70,71]. The current data suggested that in regions where HA coating was no longer present, the coating was replaced by remodeled bone leading to direct contact along the CoCr surface of the implant. It appears, therefore, that the long-term implant status of integration was not affected by the HA-coating loss, consistent with data presented in other studies [68-71].

This study was limited in the use of specimens from only three donors, which were all women over 70 years of age. The observed differences in implant-bone and HA particulates-bone interface adjacent to the dental implants, therefore, may not be representative of the human population as a whole. However, the study answered the question with the high probability that osteoclasts are the key in cellular mechanism of bone remodeling. Proposed future study would be addressing the immunochemical reactions for cathepsin K identification and may provide more reliable quantitative evaluation of the cellular mechanism analyzed in this study.

Under the conditions of this review this study suggests that a low probability for removal of Custom Osseous Integrated Implant device exists if they have been in service for 11 years. The mandibular Custom Osseous Integrated Implant device supported and retained implant prosthesis satisfactorily, served many patients who could not successfully use conventional mucosa-supported complete dentures. The results of this review suggest continued consideration of the subperiosteal implant prostheses modality.
for selected patients, particularly those with severely resorbed mandibular ridges.

These results are in line with previous published studies [68, 72-75].
9. NULL HYPOTHESES

The null hypotheses were confirmed, as follows.

1. Bone remodeling of implant/bone, implant HA coating/bone and HA particulates/bone interfaces after 11 years of functional loading showed a cell mediated mechanism of implant/bone and HA particulate/bone remodeling.

2. Cell type was confirmed by TRAP staining that multinucleated cells associated with bone remodeling mechanism, which were consistent primarily of osteoclasts.
10. CONCLUSIONS

In this study of relatively long term Custom Osseous Integrated Implant (COII) device, the central objective was to investigate histological and histomorphometrical properties related to the cellular mechanisms leading to the in vivo stability, partial stability, or instability. The stability considerations were for functional implant interfaces (until death at about 11 years post-surgery and restoration) for HA (CaP) particulate bone graft, HA (CaP) coating on alloy, and Co-Cr-Mo alloy, implant surfaces. Considering the mechanisms of bone interaction with each of the biomaterials, current results were consistent with the presence of relatively immature bone adjacent to the implants and osteoclastic remodeling of bone without signs of abnormal inflammatory reactions. The implant-bone interface, whether coated with HA (CaP), partially removed or totally removed, showed bone to implant integration. The histomorphometric analyses showed 47.31±73.1% of the total implant surface covered by HA coating after roughly 11 years in vivo. In these specimens, a direct contact between bone and implant was found, without an interposition of the graft material particles. Observation focusing on bone remodeling under conditions of previous functional loading had shown (HA)(CaP) particulate bone graft, HA (CaP) coating on alloy, and Co-Cr-Mo alloy were all well integrated and subject to bone remodeling mechanisms. The type of cellular reaction that characterized the mechanism of bone remodeling
included TRAP+ cells, on the bone surface, which was confirmed by morphology and TRAP staining to be osteoclasts. Due to a small sample size, statistical analysis was not utilized. For relative interface comparisons, implant-bone interface, regardless of remaining HA coating as well as HA particulates-bone interface were surrounded by osteoclasts at different magnitudes. There was trend among all materials observed, where HA particulates showed the most osteoclast activity, followed by HA coated implant surfaces. The current data showed that in regions where HA coating was no longer present, the coating was replaced by remodeled bone leading to direct contact along the Co alloy surface of the implant.

The present study showed that there COII devices, resulted in successful clinical survival for more than 11 years, as reported by Dr. Martin. Furthermore, the study provided new insight into the properties of bone surrounding HA-coated implants and HA particulates, as well as the function of osteoclasts in long term implant to bone integration. Within the limitation of this study, (3 female patients post mortem, ages 74-94), the observed implant to bone and HA particulate to bone properties, may not be representative of the human population as a whole. In future studies, it may be possible to address the question of sample size, since Dr. Martin has been placing COII devices in his patients since 1995.
LIST OF REFERENCES


3. Edelstein BL, Douglass C. Dispelling the myth that 50 percent of the U.S. schoolchildren have never had a cavity. Public Health Reports 1995;110(5):522–530


APPENDIX A

IRB APPROVAL FORMS
### 1. Today's Date

| 12/08/2011 |

### 2. Principal Investigator (PI)

<table>
<thead>
<tr>
<th>Name (with degree)</th>
<th>Jack E Lemons Ph.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Department</td>
<td>Prosthodontics</td>
</tr>
<tr>
<td>Office Address</td>
<td>1919 7th Ave S SDB #615</td>
</tr>
<tr>
<td>E-mail</td>
<td><a href="mailto:jlemons@uab.edu">jlemons@uab.edu</a></td>
</tr>
<tr>
<td>Blazer ID</td>
<td>jlemons</td>
</tr>
<tr>
<td>Division (if applicable)</td>
<td>Biomatertials</td>
</tr>
<tr>
<td>Office Phone</td>
<td>205-934-9206</td>
</tr>
<tr>
<td>Fax Number</td>
<td>205-975-6108</td>
</tr>
<tr>
<td>Contact person who should receive copies of IRB correspondence (Optional)</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Laura Merrill</td>
</tr>
<tr>
<td>Phone</td>
<td>205-934-5022</td>
</tr>
<tr>
<td>E-Mail</td>
<td><a href="mailto:lmerrell@uab.edu">lmerrell@uab.edu</a></td>
</tr>
<tr>
<td>Fax Number</td>
<td>205-975-6108</td>
</tr>
</tbody>
</table>

### 3. UAB IRB Protocol Identification

<table>
<thead>
<tr>
<th>3.a. Protocol Number</th>
<th>X050823001</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.b. Protocol Title</td>
<td>Analyses of In Situ and Explanted Surgical Implant Devices</td>
</tr>
</tbody>
</table>

### 3.c. Current Status of Protocol—Check ONE box at left; provide numbers and dates where applicable

- Study has not yet begun: No participants, data, or specimens have been entered.
- In progress, open to accrual: Number of participants, data, or specimens entered:
- Enrollment temporarily suspended by sponsor
- Closed to accrual, but procedures continue as defined in the protocol (therapy, intervention, follow-up visits, etc.): Number of participants receiving interventions:
- Closed to accrual, and only data analysis continues: Number of participants in long-term follow-up only:
- Date closed: Total number of participants entered:

### 4. Types of Change

Check all types of change that apply, and describe the changes in Item 5.c. or 5.d. as applicable. To help avoid delay in IRB review, please ensure that you provide the required materials and/or information for each type of change checked.

- Protocol revision (change in the IRB-approved protocol)
  - In Item 5.c., if applicable, provide sponsor's protocol version number, amendment number, update number, etc.

- Protocol amendment (addition to the IRB-approved protocol)
  - In Item 5.c., if applicable, provide funding application document from sponsor, as well as sponsor's protocol version number, amendment number, update number, etc.

- Add or remove personnel
  - In Item 5.c., include name, title/degree, department/division, institutional affiliation, and role(s) in research, and address whether new personnel have any conflict of interest. See “Change in Principal Investigator” in the IRB Guidebook if the principal investigator is being changed.

- Add graduate student(s) or postdoctoral fellow(s) working toward thesis, dissertation, or publication
  - In Item 5.c., (a) identify these individuals by name; (b) provide the working title of the thesis, dissertation, or publication; and (c) indicate whether or not the student's analysis differs in any way from the purpose of the research described in the IRB-approved HSP (e.g., a secondary analysis of data obtained under this HSP).

- Change in source of funding; change or add funding
  - In Item 5.c., describe the change or addition in detail, include the applicable OGCfA tracking number(s), and provide a copy of the application as funded (or as submitted to the sponsor if pending). Note that some changes in funding may require a new IRB application.
Add or remove performance sites
In Item 5.c., identify the site and location, and describe the research-related procedures performed there. If adding site(s), attach notification of permission or IRB approval to perform research there. Also include copy of subcontract, if applicable. If this protocol includes acting as the Coordinating Center for a study, attach IRB approval from any non-UAB site added.

Add or change a genetic component or storage of samples and/or data component—this could include data submissions for Genome-Wide Association Studies (GWAS)
To assist you in revising or preparing your submission, please see the IRB Guidebook for Investigators or call the IRB office at 934-3789.

Suspend, re-open, or permanently close protocol to accrual of individuals, data, or samples (IRB approval to remain active)
In Item 5.c., indicate the action, provide applicable dates and reasons for action; attach supporting documentation.

Report being forwarded to IRB (e.g., DSMB, sponsor or other monitor)
In Item 5.c., include date and source of report, summarize findings, and indicate any recommendations.

Revise or amend consent, assent form(s)
Complete Item 5.d.

Addendum (new) consent form
Complete Item 5.d.

Add or revise recruitment materials
Complete Item 5.d.

Other (e.g., investigator brochure)
Indicate the type of change in the space below, and provide details in Item 5.c. or 5.d. as applicable. Include a copy of all affected documents, with revisions highlighted as applicable.

5. Description and Rationale
In Item 5.a. and 5.b, check Yes or No and see instructions for Yes responses.
In Item 5.c. and 5.d, describe—and explain the reason for—the change(s) noted in Item 4.

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>5.a. Are any of the participants enrolled as normal, healthy controls?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>If yes, describe in detail. Item 5.c. how this change will affect those participants</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>5.b. Does the change affect subject participation, such as procedures, risks, costs, location of services, etc.?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>If yes, FAP-designated units complete a FAP submission and send to <a href="mailto:fap@uab.edu">fap@uab.edu</a>. Identify the FAP-designated unit in Item 5.c. For more details on the UAB FAP, see <a href="http://www.uab.edu/fap">www.uab.edu/fap</a>.</td>
</tr>
</tbody>
</table>

5.c. Protocol Changes: In the space below, briefly describe—and explain the reason for—all change(s) to the protocol.

Student to evaluate specimens for master of science project. Dave Korie added in SIPB.

6.d. Consent and Recruitment Changes: In the space below, (a) describe all changes to IRB-approved forms or recruitment materials and the reasons for them; (b) describe the reasons for the addition of any materials (e.g., addendum consent, recruitment); and (c) indicate either how and when you will reconsent enrolled participants or why reconsenting is not necessary (not applicable for recruitment materials).

Also, indicate the number of forms changed or added. For new forms, provide 1 copy. For revised documents, provide 3 copies:
* a copy of the currently approved document (showing the IRB approval stamp, if applicable)
* a revised copy highlighting all proposed changes with "tracked" changes
* a revised copy for the IRB approval stamp.

Signature of Principal Investigator: [Signature]
Date: 12/09/11
Notify IRB of Contact Changes

(Please type: In MS Word, highlight the shaded box and replace with your text. Double-click checkboxes to check/uncheck.)

- This form may be used to notify the IRB of
  (a) departure of persons who will no longer serve as an IRB contact;
  (b) staff replacements or additions who will serve as IRB contacts; and
  (c) additions of persons to be notified when approval of a protocol is renewed by the IRB.
- All three categories may be submitted on a single form, but a separate form must be used for each Principal Investigator (PI) affected by changes.

Please remove the following individual as an IRB contact for the protocol(s) listed.

Name to Remove: **Dave Kojic**  
E-mail: darko@uab.edu  
Blazer ID (if applicable): **Darko**  
Telephone: 205-996-5746

☐ All protocols of PI named — OR —  
☒ The protocol(s) listed below.

1. Title: **Analyses of In Situ and Explanted Surgical Implant Devices**  
IRB Protocol Number: **X050823001**

2. Title:  
IRB Protocol Number: _____

Date Removal Becomes Effective: _____

Please add the following individual as an IRB contact for the protocol(s) listed.

Name to Add: **Laura Merrill**  
E-mail: l.merrill@uab.edu  
Blazer ID (if applicable): **Lwoodruf**  
Telephone: 205-934-5022

☐ All protocols of PI named — OR —  
☒ The protocol(s) listed below.

1. Title: **Analyses of In Situ and Explanted Surgical Implant Devices**  
IRB Protocol Number: **X050823001**

2. Title:  
IRB Protocol Number: _____

Date Addition Becomes Effective: **12/9/11**

Please send copies of renewal notices for the IRB protocols listed below to the following individuals.

Name: **Robbie Burrell**  
E-mail: rburrell@uab.edu

☐ All protocols of PI named — OR —  
☒ The protocol(s) listed below.

1. Title: **Analyses of In Situ and Explanted Surgical Implant Devices**  
IRB Protocol Number: **X050823001**

2. Title:  
IRB Protocol Number: _____

PI Name: **Jack E. Lemons, PhD**  
PI E-mail: lemons@uab.edu  
Date: **12/9/11**  
PI Telephone: 205-934-9206

PI Signature: 

Contact Change Form (2)  
3/30/07

Page 1 of 1
FOR IRB USE ONLY

☐ Received & Noted  ☑ Approved Expedited  ☐ To Convened IRB

[Signature (Chair, Vice-Chair, Designee)]  [Date]

D.O.A. 8-5-11

Change to Expedited Category  Y / N / NA

*No change to IRB’s previous determination of approval criteria at 45 CFR 46.111 or 21 CFR 58.111