Peri-implant Indices of Remodeling as a Response to Mechanical Loading

THESIS

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By

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Abstract

**Background:** The current study aims to detect peri-implant tissue changes that may occur due to bone and soft tissue remodeling as a response to mechanical loading.

**Material and methods:** Systemically healthy, non-smoking adults with a single posterior edentulous space with intact proximal natural teeth were recruited. Inclusion criteria were: adult patient, systemic health, periodontal health, single tooth posterior edentulous space, non-smoking, no grafting needed, and ability to consent to treatment. Exclusion criteria included presence of parafunctional habits and implants placed with significant angle precluding screw retained implant crowns. Peri-implant tissue changes were recorded prior to crown delivery and 1, 3, 6 and 12 weeks in function. Clinical parameters were: plaque and gingival indices (Pl and GI), Resonance Frequency Analysis (RFA), and crevicular fluid (CF) samples of the implant and adjacent teeth. CF volume was determined using a calibrated electronic volume quantification unit. Samples were stored at -80°C. Multiplex assay was performed for cytokine detection. Polymerase Chain Reaction was used to detect the presence of eight putative microflora. Data was analyzed using multiple model regression analysis corrected with Bonferroni adjustment. Correlation analysis was conducted by calculating Spearman correlation coefficient, statistical significance was accepted at p<0.05.
**Results:** Sixteen patients were recruited and fifteen completed the study. Mean healing time prior to loading was 6.1±0.3 months. Implant Stability Quotient (ISQ) increased from 76.5±1.7 to 83.7±1.7 units (P<0.001). Peri-implant plaque accumulation was statistically lower than adjacent periodontal plaque accumulation at baseline (P=0.02) and this difference between groups stayed significant throughout the observation weeks (P<0.004). Peri-implant gingival index was statistically lower compared to adjacent periodontal gingival index before loading and at 1 week following loading (P<0.05). No difference between implant and adjacent tooth sites for GI at follow-up appointments (P>0.05). Peri-implant GI increased observed by 12 week was statistically significant compared to baseline (P=0). Gingival crevicular fluid (GCF) was statistically higher than peri-implant crevicular fluid (PICF) prior to loading (P=0.03). PICF increased following loading and was significantly greater than GCF at 1, 3, 6 and 12 weeks (P<0.04). Post-loading peri-implant crevicular fluid was statistically higher than pre-loading crevicular fluid when compared to baseline value (P=0.004). No significant differences in periodontal and peri-implant probing depths or the keratinized tissue width throughout the study (P>0.05).

Cytokines: PDGF, IL-4, IL-6, IL-7, IL-10, IL-12, IL-17, Eotaxin, FGFb, IFNγ and TNFα were expressed at detectable but low levels at both PICF and GCF samples with no significant differences throughout the observation period. IL-1β, IL-8, G-CSF, IP-10 and VEGF presented higher expression levels in gingival crevicular fluid samples compared to PICF samples prior to loading with no statistically significant difference. Following one week of loading, there was approximately a two fold increase in peri-implant crevicular fluid IL-1β, IL-8 and MIP-1b levels although the differences were not statistically significant. VEGF and MCP-1 levels showed a
trend towards increased expression at one week (P>0.05). Other cytokines, G-CSF and IP-10, were expressed at higher levels at both PICF and GCF samples following 3 weeks of loading compared to before loading values. The differences were not statistically significant (P>0.05). Microbiology data showed the presence of certain putative periodontal pathogens throughout observation period in a transient fashion.

**Conclusion:** Within the limits of this study, it appears that mechanical loading following a traditional osseointegration period has significant effect on implant stability and peri-implant clinical indices used to evaluate soft tissue healing and health. However, the expression of pro-inflammatory cytokines and the presence of certain putative periodontal pathogens show significant variations among individuals.
Dedication

This document is dedicated to my family. They had to sacrifice a lot to allow me to complete this project.
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CHAPTER 1: INTRODUCTION

Successful outcome of implant placement is dependent on deposition of bone on the implant surface during the healing stage. Although not well documented, early implant failures have been reported to occur at rates ranging from 3 to 20 percent. [1] [2] [3] Failed implants have detrimental effects on the patient’s comfort, esthetics, and oral health. Dental implant therapy is a time consuming, and financially demanding process. Therefore, it is imperative that the initial implant placement outcome is a success. Osseointegrated implants have been reported to have positive long-term clinical results. [2] [3] Implant stability is a key characteristic of successfully osseointegrated implants. [1] [4] [5]

Implant placement requires surgical manipulation of the jaw bone; accomplished with an osteotomy procedure resulting in trauma to the bone. Osseointegration then begins with an acute inflammatory response to the surgical trauma. A blood clot forms between newly sectioned bone and newly introduced implant device. The implant surface functions as a scaffold controlling cell migration. Inflammation is a necessary part of the early phase of wound healing. Previous work (Emecn-Huja MS thesis 2011) demonstrates a fast response to injury and physiologically resolves within one to two weeks of post-operative healing in humans. The current study aimed to determine whether similar peri-implant inflammatory responses are detectable following loading.
Changes in implant stability following occlusal loading were also investigated by using resonance frequency analysis (RFA).

**Wound healing during early phases of osseointegration**

In order to achieve osseointegration [6], bone and soft tissue surrounding a newly placed dental implant device must heal properly. Peri-implant bone healing follows the general bone healing cascade of events which includes: hemorrhage, clot formation, an immunologic response, immature or woven bone formation, and remodeling [7]. This process results in the removal and repair of surgically damaged bone and subsequent osseointegration of a dental implant. Similarly, soft tissue healing follows the general sequence of: trauma, hemorrhage, clot formation, cellular activation, granulation tissue formation, scar formation, and final connective tissue remodeling [7]. The final product is a rigidly bound bone-to-implant surface and a mucosal seal. The process of osseointegration of dental implants is divided into early and late phases. The early phase includes healing around an implant immediately following its surgical placement until loading. The late phase includes healing or remodeling that occurs after the implant is loaded.

Davies [8] divided early phases of wound healing at bone level into three stages: osteoconduction, *de novo* bone formation, and bone remodeling. The *de novo* bone formation around a newly placed implant can be divided into two categories: contact osteogenesis (implant surface apposition of bone) and distant osteogenesis (bony surface apposition of bone). Oral mucosal epithelium healing follows the same basic wound healing mechanism as epidermal tissue. As described by Wikesjo, [9] injury to the
mucosal epithelium results in bleeding. Within seconds, plasma proteins and tissue proteins are released into the wound, initiating the coagulation cascade. Minutes later a fibrin clot is formed providing wound hemostasis. Phagocytic cells (neutrophils dominate early and then macrophage in the later stages) begin to debride the area of damaged tissue and foreign material. The presence of phagocytic cells stimulates cell activation and migration to the wound area. Epithelial cells begin to migrate across the top of the fibrin network and within three to four days they seal the wound from the oral environment. Within the first two to three days following tissue injury, fibroblasts begin producing a disorganized collagen network and endothelial cells begin capillary formation. As a result, granulation tissue is formed. This granulation tissue undergoes a maturation and remodeling process culminating with the formation of a scar and eventually normal oral mucosa. Dental implants placed following a one-stage (non-submerged) surgical protocol allow the investigation of early phases of peri-implant wound healing during osseointegration by creating a clinical study model. [9]

Soft tissue around an unloaded one stage implant, when fully healed, exhibits similar characteristics as a natural tooth periodontal mucosa. Healthy peri-implant soft tissue characteristics include: keratinized gingiva, 0.23 to 0.65 mm of non-keratinized sulcular epithelium, 1.16 to 1.71 mm of junctional epithelium, and 0.79 to 1.39 mm of collagen rich (scar like) connective tissue. Together these components comprise the peri-implant biologic width, about 2.84 to 3.01 mm [10] [11] [12] [13]. It’s important to note that the peri-implant junctional epithelium is attached to the implant surface via hemidesmososes, but not to the extent as seen with natural teeth [14] [15] [16] and the
collagen fibers of the connective tissue layer run parallel to the implant surface with no apparent connection to the implant surface [17].

**Bone Response to Load**

Bone is a dynamic tissue, meaning bone is continually changing or remodeling in response to the forces it receives. In the early 1800’s Bougery, and Bell separately documented the unique internal structure of long bones. [18] [19] Their concept of bone architecture were confirmed and reported independently by von Meyer. [19] [18] [20] Later in the 1800’s an engineer named Culmann related the boney architecture of a femoral head, to the trajectories used to create the “Culmann Crane.” [19] [21] Julius Wolff added to his predecessors work and presented the laws of bone remodeling which is currently known as “Wolff’s Law” or “The law of transformation of the bone.” [18] [19] [22] [23] Later Roux added the principle of functional adaptation. [18] [19] [24] In summary, bone responds to stress and strain by modeling and remodeling its boney architecture.

Bone adjacent to a dental implant’s surface may experience bone remodeling in response to forces generated during function [18]. Stanford and Brand [18] review the concepts of bone remodeling around dental implants. The bone remodeling process appears to exhibit an “on” and “off” response, known as the mechanostat theory. [25] Frost presented the mechanostat theory as part of bone remodeling mechanism. [25] His theory states that activation or deactivation of the bone remodeling process is dependent on the amount and frequency of force applied to the bone. This theory in turn suggests that there is a minimal strain level required in order to initiate bone remodeling. Frost
claims that strain levels of 50 - 200 μ-strains result in disuse atrophy of bone. [25] Also, 2000 μ-strains is the “minimum effective strain” to induce bone remodeling. [26] It is thought that mechanostat mechanism is controlled in part by the process of mechanotransduction. [18]

Mechanotransduction is the process by which bone translates mechanical stress into a biologic response of remodeling or modeling [18]. Stress on bone results in an increase in intra-boney fluid pressure and movement. The resultant fluid changes are recognized by osteocytes. In turn, osteocytes respond by producing bone remodeling mediators which regulate activation and coupling of osteoclastic and osteoblastic activity, also known as the basic multicellular units (BMUs) of bone. [27] [28] [29] [30] [31] [32] [33] The induction of newly forming bone is closely coupled to osteoclast activity, which allows bone to remodel as an adaptation to the forces which it receives. [28] [29]

Occlusal forces applied to an implant restoration are transferred to the bone via the implant-bone interface and affect bone in varying degrees of strain. [18] [34] [35] [36] [37] An implant and its restorative components act as a single entity if the screw preload and passivity of the restoration are adequate. [38] In vitro studies, using finite elemental analysis and photoelastic studies, predict an even distribution of force into the adjacent peri-bone when an axial load is applied to a vertically positioned implant. The resultant peri-bone strains are greatest in the coronal bone adjacent to an implant followed by apical peri-implant bone. Bone strain distribution between the implant-bone interface is affected by a variety of factors. Some of these factors include: quantity of bone, quality of the bone, bone height, implants’ spatial location within bone, the implant
size, and the implant angulation. [34] [35] [36] [37] [39] [40] [41] Peri-implant bone strain as a result of occlusal load induces peri-implant bone remodeling. Animal studies indicate that loaded implants result in greater peri-implant bone density. [42] Static load studies report rearrangement of osteocytes adjacent to loaded implants [43], greater remodeling in peri-implant bone with loaded implants [43], cancellous bone trabeculae remodeling based on strain patterns [44], and greater bone density during first weeks of loading especially in the coronal part of the implant site. [45] [46] [47] Also, Turner, using a rat model, demonstrated that bone remodeling response increases by increasing the amount and the frequency of mechanical stress. [27]

Peri-implant soft tissue characteristics after loading remain similar to natural periodontal tissues and unloaded peri-implant structures. [12] [48] [49] Others report slight changes in the junctional and sulcular epithelial dimensions, suggesting an increasing in length of the junctional epithelium [12].

**Clinical parameters used to evaluate peri-implant tissue health**

Implant osseointegration success has always been the primary goal of dental implant therapy. Success and failure criteria have been significantly discussed and debated. [1] [4] [5] At present, many schools of thought have accepted the success and failure criteria developed by Albektsson and Zarb. [4] [5] However, in specific cases, implants may clinically present with characteristics somewhere in between the “successful” and “failed” criteria. These have been described as failing or ailing
implants. Monitoring and determining peri-implant disease progression is an active area of research.

Conventional methods used for evaluation of peri-implant health include peri-implant probe depths (PD), some form of recording for gingival inflammation, plaque accumulation, radiographic evaluation, peri-implant crevicular fluid (PICF) evaluation, and recording implant stability. [1] [5] All methods are helpful but possess inherent limitations especially regarding implant health prognostication. [1] However, PICF characterization and monitoring implant stability with resonance frequency analysis (RFA) have shown some promise. The following describes peri-implant probe depths, gingival bleeding, radiographic evaluation, implant stability using periotest and RFA, and crevicular fluid as possible diagnostic tools.

**Peri-implant Probe Depths**

Peri-implant probe depths when accurately recorded over time are helpful in monitoring peri-implant inflammation and health. [1] However, due to inherent differences in peri-implant gingival tissues [50] [51], difficulty in controlling probing angulation and forces [52] [53], and varying soft tissue factors [1] limits its use in accurately determining peri-implant health and prognostication.

**Peri-implant Gingival Bleeding**

Gingival bleeding or inflammation indices are used to monitor implant health. Bleeding has been correlated with peri-implant disease. [1] [51] Conversely, peri-
implant bleeding on probing has been reported to occur with healthy and diseased peri-
implant gingival tissues. [50] [51] [54]

**Radiographic Evaluation**

Radiographic evaluation is the most widely used method of clinically monitoring peri-implant bone support [55] [56] [57] Limited resolution and lack of three dimensional analyses prevent the panoramic radiograph from being used for fine measurements. [57] Peri-apical radiographs obtained with proper paralleling technique in conjunction with opaque spheres (BBs) of know dimension for measurement correction provide a minimally distorted fine detail analysis of peri-implant bone. They are often used to measure crestal bone height changes over time. [55] [57] Some limitations exist with peri-apical radiography to evaluate peri-implant bone. These factors include: human error in reading the radiograph, angulation repeatability, two dimensional view of the implant, and limited resolution. [1] Cone beam CT is the most accurate method of evaluating integration of dental implants. It provides a better one to one scale and a three dimensional representation of the implant site. However, the higher exposure to radiation, compared to conventional dental radiography, and increased cost limit their routine usage as a diagnostic tools [58] [59].

**Implant Stability**
Non-invasive techniques for evaluating implant stability include Periotest® and resonance frequency analysis. Periotest® utilizes mechanical tapping of a tooth or implant. It is designed to quantify the dampening effect of a periodontal ligament [60]. When placed over an implant surface and activated, it provides 16 consecutive taps at a controlled force to the implant surface. Stability determination is based upon the duration of each tap on the implant surface. This recording is given a numeric value, the manufacture reports a range of -8 to 50. A smaller number signifies greater stability. It is reported that implant stability recording produce numeric values in the range of -5 to 5. [61] [62]. If an implant exhibits any movement within the bone, the contact duration of each Periotest® tap is increased and results in a larger numeric value. Due to relatively poor sensitivity, poor resolution, and its susceptibility to operator error [63] [62] new technology has been developed.

Resonance frequency analysis (RFA) was introduced as a possible method to determine implant stability by Meredith during the 1990’s. [64] The concept is based on recording the resultant vibration/movement of a transducer attached to an implant when stimulated. Originally, an L-shaped transducer was attached to an implant and excited with a known frequency. The L-shaped transducer was attached to a frequency analyzer which recorded the transducers vibration response to the stimulation. Recordings were in Hz; the more rigid the implant-bone interface was the larger the Hz recording. [63] This was interpreted as an increase in implant stability. This technology was mainly used for research. More recently, RFA was developed into a clinical device known as the

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a Periotest Medizintechnik Gulden e. K., Eschenweg 3, 64397 Modautal, Germany
Osstell®\textsuperscript{b}. The Osstell® system utilizes a stimulator, a Smart Peg\textsuperscript{c}, and recording device. The Smart Peg\textsuperscript{c} consists of an aluminum peg with a screw at one end and a cylindrical magnet on the other end. To use, one first attaches the Smart Peg to an implant, then places the Osstell® wand within 2 mm of the magnet to activate the system. The wand stimulates the magnet on the Smart Peg with a magnetic pulse. The magnet vibrates and the Osstell® wand records the degree of vibration in Hz. The Osstell® unit then converts the Hz recording into a numeric value of 1-100 which is referred to as the implant stability quotient (ISQ). The larger the ISQ value recorded indicates the more stable the implant-bone interface. [65]

RFA has been used extensively to evaluate implant stability during post implant surgery healing. [66] [67] [68] [69] [70] [71] [72] [73] [74] [75] A proposed acceptable ISQ reading at the time of implant placement is generally higher than 50 ISQ. [68] [69] [74] A decrease in ISQ recordings is to be expected during the third and fourth weeks following implant surgery. By week six, original ISQ readings are normally re-established. [69] [71] [73] [74] [75] Early implant failure ISQ values appear to decrease during the first two weeks post implant surgery and continue to decrease in the subsequent weeks. ISQ readings in the 40s at weeks 2-4 have been reported on early failure implants. [69] [70] [72] [73] [74] [75] However, some implants that exhibit lower ISQ recordings which are left untouched for a period of time may recover and become successful. [75] Factors that affect initial ISQ recordings include: amount of cortical bone present, method of site preparation, amount of implant above crestal bone,
and implant shape. [62] [68] [70] [72] [74] ISQ is not affected by implant length. [62] [74] Implants placed in higher quality bone, rich in cortical bone, present less variation in ISQ readings during healing when compared to implants placed in lesser quality bone. [62] [70] [72] ISQ changes as a result of mechanical loading of the implant have been investigated in limited number of studies. [72] [73] [74] [75] Studies indicate that there might be slight increases in ISQ readings once an implant is loaded. Further research is needed to better correlate ISQ and loaded implants.

**Peri-implant Crevicular Fluid (PICF)**

Gingival crevicular fluid (GCF) arises, in healthy gingival tissues, from the junctional-epithelium cells of the gingival attachment apparatus. Alfano described crevicular fluid as: osmotically-modulated, pre-inflammatory exudates or transudate pulled from the cells via an osmotic gradient created by the bacterial by-products within the gingival sulcus. [76] GCF flow rate in healthy gingiva, as described by Goodson, is three to eight µl per hour. [77] GCF possesses similar characteristics as plasma, interstitial fluid, and serum [78] [79] [80] with minor modifications; such as greater concentrations of sodium and potassium. [78] [81]

When bacterial presence increases, resultant by-products will penetrate the junctional epithelium and underlying basement membrane via inter-cellular channels. These by-products result in an inflammatory response. A resultant gingival fluid exudate is generated from the capillary plexus subadjacent to the gingival basement membrane. The exudate passes through the gingival basement membrane and through enlarged inter-
cellular channels of the junctional epithelium and into the gingival crevice. Subsequently, GCF volume and flow rate increase exponentially, 10 to 70 times, compared to that of healthy non-inflamed gingival tissue. [76] [82] [77]

Healthy GCF contains a variety of molecular components and cells. Contents include host derived cells, biologic molecules, bacteria, and bacterial by-products. Sixty-five to 100 different components of GCF have been evaluated as markers to monitor inflammation and disease of the periodontal apparatus. [83] [84] These markers can be classified as host-derived enzymes, inflammatory mediators, and tissue-breakdown products. [83] Many markers have been shown to increase in the presence of inflammation and periodontal disease with a subsequent decrease after receiving periodontal therapy. Their presence may be affected by genetics, pocket depth, extent of inflammation or periodontal disease. [85] [86] [87] [84] Notable pro-inflammatory markers include: prostaglandin E2 (PGE2), elastase, lysosomal β-glucoronidase, alkaline phosphatase, cathsepsin β, collagenases or metal metalloproteinases (MMPs), interleukin-8 (IL-8), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), osteocalcin, proteoglycans, fibronectin, epidermal growth factor (EGF), transforming growth factor-alpha (TGF-α), immunoglobulin-gamma (IgG), immunoglobulin-alpha (IgA) complement components, and others. [82] [88] [89] [90] [91] However, it is difficult to use the markers to predict the potential progression of periodontal disease. [83] [91]

Peri-implant crevicular fluid (PICF) volume and composition of healthy implants is similar to natural teeth. [92] [93] [94] Sulcular depth, implant size, and one stage placement surgeries may increase the fluid volume. [95] [96] PICF volume increases
during implant loading and may remain elevated without increases in gingival inflammation. \[96\] \[97\] A significant increase in PICF volume occurs in response to peri-implant inflammation. \[95\] \[98\] \[99\] PICF fluid volume decreases subsequent to peri-implant therapy. \[100\] It is thought that PICF volume can be used to help identify peri-implant inflammation.

Nowzari \[101\] \[102\] observed similarities in PICF composition when compared to GCF of natural teeth. However, he noted a general increase in pro-inflammatory cytokine concentrations in PICF; suggesting possible implant ion release into the peri-implant tissues may stimulate small changes in cytokine composition. Similar to natural teeth, PICF contains many biological molecules. Possible biological markers include: pro-inflammatory cytokines, enzymes, and catabolic metabolites. \(\text{(Table 7)}\) These molecules may provide a means to better monitor implant health and disease progression. Pro-inflammatory molecules found and studied in the dental literature include: interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), and prostaglandin E-2 (PGE2). Some of the PICF enzymes present in the dental literature include: matrix metalloproteinases (MMPs), neutral protease (NP), neutrophil elastase (NE), myeloperoxidase, β-glucoronidase, cathepsin, and elastase. Also, several extracellular degradation products have been studied in the dental literature, including: chondroitin-4-sulfate, hyaluronic acid, and carboxytermial telopeptides. \[82-112\]

The knowledge on remodeling around dental implants following mechanical loading is limited in the dental literature. The purpose of the study was to determine peri-implant characteristics as a response to loading.
The working hypothesis states that dental implant loading following conventional osseointegration period affects implant stability, crevicular fluid volume and content.

The specific aims include:

1- Identify peri-implant soft tissue clinical changes following implant loading.
2- Determine crevicular fluid volume changes with respect to implant loading.
3- Determine if RFA can detect changes in implant stability in response to implant loading.
4- Detect presence of pro-inflammatory wound healing mediators within peri-implant crevicular fluid prior to and following implant loading.

The results of this study may help to better understand possible clinical and biological markers of remodeling that occur in peri-implant tissues following mechanical loading of a single osseointegrated dental implant. The study may be the first to investigate soft tissue changes during early phase of remodeling immediately following mechanical loading by using a clinical prospective observational study model.
CHAPTER 2: MATERIAL AND METHODS

Study Population Study Design

The study design was a prospective longitudinal observational cohort study. Subjects were recruited from the Graduate Periodontal Clinic at The Ohio State University between November 2010 and December 2011. The study protocol and informed consent forms were approved by the Institutional Review Board of the Ohio State University. A written consent form was obtained from all participants before entry to study (OSU IRB Protocol #2007H0261). Recruited patient presented with a need for a one stage single tooth implant placement surgery in a posterior sextant. When a patient met the inclusion criteria he/she received a comprehensive dental exam in the Advanced Prosthodontic Residency Clinic at The Ohio State University.

The inclusion criteria included: 1) adult patients; 2) single edentulous posterior space with adjacent natural teeth; 3) non-smokers; 4) periodontal and systemic health; 5) possess the ability and were willing to provide informed consent for the study protocol; and 6) surgical placement allowed for a screw retained implant restoration. Exclusion criteria were: 1) patients with untreated periodontal disease; 2) patients with other dental restorative needs including the presence of broken/decayed teeth; 3) implants placed with two-stage protocol; 4) grafted implant sites; 5) patients with major occlusal deficiencies; 6) patients with parafunctional habits; 7) patients with systemic diseases affecting their
oral health, eg; uncontrolled diabetes; use of NSAIDs; 8) pregnant women; 9) unable or unwilling to provide informed consent form. Exit criteria were: 1) voluntary withdrawal; 2) non-compliance with study protocol; 3) development of systemic or oral disease that are in the exclusion criteria and 4) implant placement precluding a screw retained restoration. The study design consisted total of nine visits. Figure 1 is a flow chart presenting different phases of the study protocol.

Initial Comprehensive Oral Exam and Details on Surgical/Restorative Phases of Treatment

At the initial visit with the Advanced Prosthodontic Residency Clinic, a comprehensive dental exam was performed; consisting of an extra- and intra-oral head and neck soft tissue examination, TMJ examination, and dental examination. The dental examination included appropriate radiographs, occlusal evaluation, individual tooth evaluation, photos, and diagnostic records. Diagnostic records included maxillary and mandibular alginate\(^d\) impressions with stock rim lock metal trays, three leaf gauge\(^e\) centric relation records\(^f\), and a facebow record\(^g\) with polyvinyl siloxane\(^h\). Casts were made using type III dental stone, duplicated with the same type of stone, and mounted in a semi-adjustable articulator using the previously mentioned records. Pathology and disease noted during the exam were explained to the patient and solutions were proposed.

\(^a\) Jeltrate\(^a\) DENTSPLY International Inc. General Counsel Office World Headquarters Susquehanna Commerce Center 221 West Philadelphia Street, York, PA 17405-0872
\(^b\) Leaf Gauge Panadent\(^b\) 580 S. Rancho Ave. Colton, CA 92324
\(^c\) Aluwax™ ALUWAX DENTAL PRODUCTS COMPANY P.O. Box 87, Allendale, MI 49401
\(^d\) Slidematic Facbow® Whip Mix Corporation 361 Farmington Avenue P.O. Box 17183, Louisville, KY 40217
\(^e\) Memorreg®2 Heraeus Kulzer 300 Heraeus Way, South Bend, IN 46614
Each patient was given the opportunity to return to their general dentist to address the noted concerns, if they so desired.

**Diagnostic Planning and Surgical Guide Fabrication**

Duplicate casts were obtained; two were used to fabricate surgical guides (Figure 1); one set was mounted in a semi-adjustable articulator and used for a full contour diagnostic waxing of the missing tooth. The tooth was waxed to ideal contour and articulation; duplicated in stone via an alginate impression and dental stone. The duplicate cast was used as a template to fabricate the implant surgical guides. A clear ethylene-vinyl copolymer 0.080 inch thermoplastic material\(^1\) was molded over the waxed tooth duplicate cast using a vacuum former machine\(^1\) providing a clear matrix of the patient’s teeth. The matrix was trimmed using a heat knife to cement enamel junctions of two teeth mesial and distal of the proposed implant site. The matrix was carefully removed from the cast. The border extensions were lightly heated with a torch and placed on and off the casts repeatedly to ensure ease of removal and insertion during surgery. The borders were then smoothed and polished. This process was repeated to create two clear vacuum formed matrices. The wax tooth was then removed from the diagnostic model and the undercuts on the adjacent teeth were blocked out with light body addition polyvinyl siloxane.\(^k\) The cast was then covered with a thin layer of

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\(^1\) 0.08 inch ethylene-vinyl copolymer clear thermo-forming sheets Henry Schein Inc. 135 Duryea Road, Melville, NY 11747

\(^j\) Vacuum forming machine 110V Henry Schein Inc. 135 Duryea Road, Melville, NY 11747

\(^k\) Addition Polyvinyl Siloxane Affinis® Wash Coltene/Whaledent AG Feldwiesenstrasse 20, CH-9450 Altstätten
petrolatum. Each matrix was filled with clear methylnethacrylate\textsuperscript{1}, confined to the location corresponding to the edentulous space; then fully seated onto diagnostic cast. Any excess clear acrylic was immediately removed and the cast with the resin filled matrix was placed into a warm water pressure pot\textsuperscript{m} for 20 minutes at 20 psi). The matrices were then removed, trimmed, and polished.

Implant location was determined by using calipers to measure the edentulous space. The ideal location was identified and marked on the cast with a blue and red colored pencil. Lines, indicating the inclination of the adjacent teeth, were drawn on the teeth mesial and distal to the implant site. The cast was placed onto a lab drill press\textsuperscript{n} and surveyed into the correct angulation and position. The first matrix was placed onto the cast and a 3.0 mm guide hole was prepared through the surgical guide. The first matrix was removed and the second matrix was placed onto the cast. A 3.0 mm guide hole was prepared in the same location and angulation as the previous guide. The first surgical guide remained with a single access hole and was to be used as the pilot hole surgical guide. The lingual wall of the second guide’s access hole was removed to provide ease of access for the surgeon to the proposed implant site. Any flash acrylic was removed and the guide was polished.

**Surgical Implant Placement**

All implants were placed in the Graduate Periodontal Clinic at The Ohio State University, by third year residents. Proper aseptic technique was followed and implants

\begin{itemize}
  \item \textsuperscript{1} Clear Ortho-jet\textsuperscript{TM} methylmethacrylate Lang Dental, 175 Messner Drive, Wheeling, IL 60090
  \item \textsuperscript{m} Aquapress\textsuperscript{TM} 5 inch Lang Dental 175 Messner Drive, Wheeling, IL 60090
  \item \textsuperscript{n} Paraskop\textsuperscript{®} M Multifunctional Milling Unit BEGO Bremer Goldschlägerei, Wilhelm-Herbst-Straße 1 D-28359 Bremen
\end{itemize}
were placed according to the implant manufacture’s protocol, using the surgical guide. All implants following a one stage protocol. The criterion for one-stage implant placement was an initial ISQ value of ≥50 and/or a torque value of 35 Ncm. Minimum healing time was 3 months, based on anatomical location and traditionally accepted osseointegration period.

**Restoration of Implant**

All restorative procedures were performed in the Advanced Prosthodontic Residency Clinic at the Ohio State University College of Dentistry by the same graduate prosthodontic resident. Crowns were fabricated by the following protocol:

1. Custom open tray polyvinyl siloxane implant impression

Custom Tray Fabrication: visible light curing dental acrylic was adapted over the full arch with 4 mm internal relief provided by two layers of base plate wax. Three vertical stops, to provide proper impression material thickness, were incorporated on non-functional cusps. All trays were cured following the manufacture’s recommendations using a light curing unit. Wax residue was removed using a laboratory steam cleaner and all borders were rounded and smoothed. The custom trays were placed back onto the patient’s diagnostic cast and the location of the implant site was identified and marked on the external surface of the tray directly above the implant site and a small 3

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a) Triad® VLC TruTray™ - Dentsply™, DENTSPLY International Inc. 570 West College Ave., York, PA 17405-0872
b) TruWax® Base plate wax - Dentsply™ DENTSPLY International Inc. 570 West College Ave., York, PA 17405-0872
c) Tiad® VLC Curing Unit - Dentsply™ International Inc. 570 West College Ave., York, PA 17405-0872
mm hole through the tray was performed. All trays then bench cured for a minimum of 24 hours prior to use on a patient.

2. Implant Impression:

The healing abutment was removed from the implant and an appropriate direct implant transfer coping was inserted and hand tightened. A peri-apical radiograph was obtained to verify complete seating of the transfer coping and to evaluate the peri-implant bone density and level. The custom tray was inserted to confirm access to the implant impression post screw through the tray. The tray was again cleansed and dried. A thin layer of polyvinyl siloxaine adhesive was applied to the internal surface of the tray and allowed to dry for 10 minutes. The patient’s mouth was dried with cotton and light body vinyl polysiloxane was syringed around the impression coping in the patient’s mouth; simultaneously heavy body polyvinyl siloxaine was syringed into the custom tray. The tray was seated appropriately in the patient’s mouth and the excess around the superior aspect of the screw was wiped away. The impression was carefully held into place until the material polymerization time was achieved. The transfer abutment screw was loosened and the impression was removed. An implant lab analog was carefully attached and hand tightened to the impression post in the impression. The

1. Caulk® Tray Adhesive - Dentsply™ DENTSPLY International Inc. 570 West College Ave., York, PA 17405-0872
2. Aquasil Ultra Light Body Regular Set Vinyl Polysiloxane Impression Material Aquasil® DENTSPLY Caulk. 38 West Clarke Ave, Milford, DE 19963-1805
3. Aquasil Ultra Heavy Body Regular Set Vinyl Polysiloxane Impression Material Aquasil® DENTSPLY Caulk, 38 West Clarke Ave, Milford, DE 19963-1805
impression was then disinfected, the healing abutment was re-inserted, and the appropriate shade was selected using the vita shade guide.

3. Crown Fabrication

Each implant impression was poured with low expansion die stone\textsuperscript{u} and mounted using hand articulation with a cast of the opposing dentition that had previously been mounted in a semi-adjustable. Casts were lightly equilibrated on the articulator prior to crown fabrication. Appropriate cast-to waxing abutments were ordered. The mounted case was then sent to a dental lab (Crown Work Dental Laboratory – Eugene, OR) for fabrication of a screw retained metal ceramic implant crown with no metal showing. The average return time from the lab was approximately three and one-half weeks.

4. Crown Insertion:

The screw retained metal ceramic crowns were tried-in. Proximal contacts were adjusted to lightly drag 8 μm shimstock foil\textsuperscript{v} to ensure complete seating of the crown. The articulation was then adjusted to be light contact in maximum intercuspation. Then a periapical radiograph was obtained. The implant crown was torqued using the manufacturers torque driver, 20 Ncm for Zimmer\textsuperscript{w} implant crowns and 15 Ncm for Straumann\textsuperscript{x} crowns. Initial insertion torque was lower than the manufactures recommendations knowing

\textsuperscript{u} Resin Rock  Whip Mix Corporation 361 Farmington Avenue, Louisville, Kentucky, USA 40209
\textsuperscript{v} Shimstock* Metal Foil Whaledent, Wash Coltene/Whaledent AG Feldwiesenstrasse 20, CH-9450 Altstätten
\textsuperscript{w} Zimmer Dental 1900 Aston Ave, Carlsbad, CA 92008-7308
\textsuperscript{x} Straumann 60 Minuteman Road, Andover, MA 01810
they crowns would need multiple subsequent removals. The access hole was then filled with a gauze strip and a visible light curing acrylic material\textsuperscript{\textcopyright}. At 12 weeks post-loading, a new periapical radiograph was obtained of the implant, with the crown in place, and crowns were torqued to 30 Ncm (Zimmer) and 35 Ncm (Straumann), respectively.

**Peri-Implant Data Collection**

Figure 1 presents an outline of the study timeline and peri-implant data collection. Samples were obtained at baseline (immediately before crown insertion) and at one, three, six, and twelve weeks post insertion. Baseline samples were recorded prior to the insertion of the implant crown. Plaque index (PI), gingival inflammation index (GI), probe depths (PD), crevicular fluid samples, and resonance frequency analysis or ISQ measurements were recorded at each data collection appointment. Peri-implant radiographs were obtained at baseline and twelve weeks as well as attached gingiva measurements.

**Plaque and Gingival Indices**

At baseline and the post insertion/loading evaluation appointments of one, three, six, and twelve weeks plaque index (PI) and gingival index (GI) \cite{110, 111, 112} for adjacent natural teeth and modified plaque index (mPI) and modified gingival index (mGI) \cite{113} for dental implant were measured and recorded.

\textsuperscript{\textcopyright} Fermit\textsuperscript{\textregistered} Ivoclar Vivadent AG, Bendererstrasse 2, 9494 Schaan, Principality of Liechtenstein
The Silness and Loe plaque index \cite{110,111,112,114} is recorded by measuring the amount of plaque present on four surfaces of a tooth; mesial, distal, buccal, and lingual. Numeric values are assigned to each surface based on the plaque detected. Based on this scoring system: 0 = no plaque is present, 1 = plaque is present at the free gingival margin, 2 = a moderate amount of plaque present within the gingival sulcus, and 3 = the presence of abundant plaque within the gingival sulcus. The four numbers are then added together and divided by four. The resultant number is the relative plaque score for that tooth. mPI is a modified version of PI for implants.

The Loe and Silness gingival inflammation index \cite{111,114} is recorded in a similar manner as the plaque index. Numeric values are assigned to each tooth surface analyzed using the following scale: 0 = normal gingiva; 1 = mild gingival inflammation, noted by slight change in color; 2 = moderate inflammation, redness, glazing, and bleeding on probing; 3 = severe inflammation, marked redness, edema, ulceration, spontaneous bleeding. The tooth surface values are added and divided by the total surfaces evaluated.

Modifications, suggested by Mombelli \cite{113}, regarding PI and GI detection around implants are related to implants lacking a physiologic gingival margin and CEJ relationship as seen with natural teeth.

**Probing Depth and Keratinized Tissue**

Probing depths were recorded on the distal buccal, mid-buccal, mesial buccal, mesial lingual, mid-lingual and distal-lingual on the implant and adjacent teeth. Probe
depths were recorded on natural teeth using a metal color coded probe\textsuperscript{z} and a plastic probe\textsuperscript{aa} was used to record probe depths around the implant.

Keratinized tissue measurements were recorded on the buccal and lingual of the implant crown at baseline and at twelve weeks. The measurement was obtained by recording the distance from the free gingival margin to the mucogingival junction using a color coded metal probe\textsuperscript{bb}.

**Gingival Crevicular and Peri-Implant Crevicular Fluid Sample Collection**

Gingival crevicular fluid (GCF) samples and peri-implant crevicular fluid (PICF) samples were collected by using sterile paper strips\textsuperscript{cc}. Crevicular fluid samples were obtained at baseline and at the subsequent one, three, six, and twelve weeks post-loading data collection appointments. Crevicular fluid was obtained using the following protocol:

1. Fluid Isolation
   
   a. The implant sextant was rinsed and dried with suction and the air water syringe. Cotton rolls were positioned in buccal vestibule adjacent to the implant, in the buccal vestibules of the maxilla to block Stenson’s duct, and sublingually. Gauze was also positioned between the tongue and the sampling area. The area was again dried with gauze and kept dry with the help of suction.

2. Crevicular Fluid Sample using Paper Strips

\textsuperscript{z} Qulix™ Color Coded Probes Hu-Friedy Mfg Co. Inc
\textsuperscript{aa} Plastic Perio Wise Probe Premer Dental Products
\textsuperscript{bb} Qulix™ Color Coded Probes Hu-Friedy Mfg Co. Inc
\textsuperscript{cc} PerioPaper Strips OraFlow Inc. 14 Threepond Road Smithtown, New York 11787
a. Paper strips were handled with sterile cotton forceps; they were only touched on the orange waxed area. A sterile paper strip was inserted into the gingival sulcus, just below the free gingival margin, of a tooth adjacent to the implant location. Each paper strip remained in place for 20 seconds and was removed with cotton forceps and placed into a calibrated electronic volume quantification unit (Periotron 8000®). The crevicular fluid volume was recorded and the paper strip was immediately transferred into a properly labeled sterile cyrotube and closed to prevent excessive evaporation; and then placed on ice. This process was repeated until four crevicular fluid samples were obtained from each implant and its adjacent teeth. (two from each tooth adjacent to implant) The same process was followed to obtain four samples of the peri-implant crevicular fluid. Samples were obtained from different locations within the gingival sulcus. Any samples with visible blood contamination with blood or saliva were discarded. All samples collected were immediately transferred to a -20°C freezer and later transferred to a -80°C freezer for long term storage.

Implant Stability Quotient Readings

Implant stability data were recorded at baseline and at one, three, six, and twelve weeks implant post-loading. Implant stability was determined using resonance frequency

<ref>Periotron Model 8000; OraFlow Inc. 14 Threepond Road Smithtown, New York 11787</ref>
analysis via the Osstell® ISQ device\textsuperscript{es}. The following protocol was used to obtain ISQ
data for each implant in the study:

1. After all the peri-implant data were collected, the healing abutment (at
baseline) or the crown (at one, three, six, and twelve weeks) were removed
and the appropriate size SmartPeg\textsuperscript{TM} was screwed into the implant and
hand tightened.

2. The Osstell® ISQ unit was activated and the wand was held within 2 mm
of the magnetic tip of the smart peg on the buccal side until a numeric ISQ
value was recorded. The wand was then placed on the lingual/palatal side
in a similar manner until a numeric ISQ value was recorded. This was
repeated two times.

3. Four ISQ values, two from the buccal and two from the lingual/palatal
were recorded.

\textbf{Laboratory Analysis of the Crevicular Fluid}

\textit{Extraction of crevicular fluid from paper strips}

Extraction of crevicular fluid was performed as following: PCR microtubes with
small holes at the bottom were prepared and sterilized. These tubes were placed into
sterile 1.5 ml Eppendorf tubes. Paper strips were separated from waxed portion by using
sterile scissor and were transferred from cyrotubes into PCR microtubes. Paper strips

\textsuperscript{es} Osstell\textsuperscript{®} ISQ Ostell AB, Gamlestadsvägen 3B, SE 415 02 Göteborg, Sweden
\textsuperscript{ff} SmartPeg\textsuperscript{TM} Ostell AB, Gamlestadsvägen 3B, SE 415 02 Göteborg, Sweden
were soaked in 200 μl cold PBS and incubated on ice for 15 minutes with occasional vortexing. Samples then were centrifuged at 13,000 rpm for 10 min. Supernatant was collected from the bottom of the tubes and centrifugation was repeated. Approximately 160 μl elution volume was recovered from each tube. Forty μl of this volume was loaded into multiplex bead-based assay plate. Remaining volume was used to detect presence of specific bacteria within newly forming peri-implant sulcus.

*Multiplex bead-based assay*

The multiplex bead-based assay was used to detect the presence and the concentration of human pro-inflammatory cytokines and growth factors within crevicular fluid samples including PDGFb, IL-1β, IL-1ra, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-17, Eotaxin, FGFb, G-CSF, IFNγ, IP-10, MCP-1, MIP-1b, TNF-α and VEGF. This technology uses digital signal processing capable of classifying polystyrine beads (microspheres) dyed with distinct proportions of red and neared florophores. The spectral addresses for different bead populations where up to hundred different detection reactions can be performed in small volume of samples. The cytokine reagent kit includes assay buffer, antibody diluent, streptavidin-PE, and a 96-well filter plate. Specific kit designated as human cytokine group (Group I) was used for cytokines and growth factors.

*Microbiology assay:*

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*Plex™ Cytokine Assay, BioRad Laboratories Inc*

*BioRad laboratories, Life Science Research, Hercules, CA*
Targeted bacterial identification was performed by polymerase chain reaction (PCR) using species specific primers in a nested amplification protocol. [115] Briefly, bacterial DNA was isolated by binding to glass beads and then eluted to Tris-EDTA buffer (pH 8). This community was used as a template to perform two round of PCR. The first round used universal bacterial primers targeted to 16S and 23S ribosomal genes. This initial amplification included part of the 16S gene as well as intergenic spacer region between the 16S and 23S genes. The second amplification used species specific forward primers along with a universal 23S reverse primer in a nested amplification using specific bacterial probes for the following known periodontal microbes (F. neviforme, G. hemolysans, T. forsythia, T. denticola, S. mitis, V. parvola, P. micros and C. gingivalis). Specific periodontal pathogens were chosen based on a recent publication [115].

Statistical Analysis

The statistical analysis was completed by a statistical consultant at the OSU Center of Biostatistics. Data were analyzed by using GraphPad Prism version 5ii and SAS version 9.2jj. Mixed Model Repeated Measures ANOVA was used to analyze the data. Sandwich estimator was used to control the correlation that might exist in the data associated with the same subject. Baseline adjusted model were fitted to the data that compare two groups. T-test were performed within the ANOVA to compare each of the two groups at that time point and compare each post time point with the baseline in that particular group, where comparison was done by applying Bonferroni adjustment method

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ii GraphPad Prism® GraphPad Software 2236 Avenida de la Playa La Jolla, CA 92037 USA
jj SAS version 9.2® SAS SAS Institute Inc.100 SAS Campus Drive Cary, NC 27513-2414 USA
to control the overall error rate. Spearman Correlation Coefficient was calculated to
determine possible correlations between various clinical parameters and cytokines. The
results were considered significant at P value ≤0.05.
CHAPTER III: RESULTS

Study population

Sixteen patients missing a posterior tooth with intact adjacent teeth were recruited for the study. Each patient underwent one-stage implant placement surgery. None of the sites were previously grafted. The mean healing time prior to loading was 6.1±0.3 months. Following the osseointegration period, all implants were restored with a screw retained metal ceramic crown. One subject was excluded during restorative phase of the treatment due to implant angulation precluding the use of a screw retained crown. One subject failed to complete the twelfth week appointment and one patient experienced an implant failure at twelfth week (maxillary premolar) appointment. The remaining thirteen subjects completed 12 week follow up study protocol.

Demographic data is presented on Table 2. The study population included seven female and eight male subjects with an overall mean age of 54±3 years. Four implants were maxillary and 11 mandibular. Four implants replaced a premolar teeth and 11 replaced molars. The mean implant diameter was 4.7±0.15 mm and the mean implant length was 12±0.3 mm. Early implant success was 100% while late implant success was 93.3% with one implant failure at 12 weeks post-loading (Table 2).

Clinical measurements

Plaque accumulation
Plaque accumulation on newly loaded implant and on adjacent teeth was recorded using PI (Figure 2). Peri-implant plaque accumulation was statistically lower than adjacent periodontal plaque accumulation prior to loading (0.3±0.13 and 0.8±0.2, respectively; P=0.02; median 0 (0-1.5) and 0.5 (0-2) respectively). The PI difference between an implant supported crown and the adjacent teeth remained statistically significant throughout the study (P<0.004). In general, plaque accumulation was well controlled with PI <1 for all surfaces, all observation periods (Figure 2).

**Gingival Inflammation**

Soft tissue characteristics were studied by recording mGI around implants and GI around adjacent teeth. Prior to loading mGI was statistically lower around implants compared to adjacent GI (Figure 3; 0.4±0.2 and 1±0.1, respectively; P<0.03); median 0 (0-1.8) and 0.9 (0.3-1.6) respectively. It remained lower at the first week following loading, (0.4±0.1 and 0.9±0.1, respectively; P<0.05); median 0.5 (0-1) and 1 (0-1.4) respectively. However, the GI difference between an implant supported crown and adjacent teeth diminished with time. The peri-implant GI approached similar levels as adjacent periodontal GI levels (Figure 3). In general, gingival inflammation was well controlled with GI<1 for all surfaces (Figure 3).

**Keratinized Gingiva**

Keratinized gingiva width measurements were recorded from the gingival margin to mucogingival junction at the mesial, mid-buccal (mid-lingual), and distal surfaces of
the implant site and reported as average measurements for the buccal and lingual surfaces. Before loading mean readings were 3.8±0.3 mm and 4.7±0.6 mm for buccal and lingual surfaces, respectively (Figure 4). Measurements at week 12 were 3.8±0.2 mm and 4.8±0.6 mm for buccal and lingual surfaces, respectively. There was no statistically significant differences between different surfaces and different observation periods (Figure 4; P>0.05).

_Probing depth_

Peri-implant and adjacent teeth periodontal probing depths were recorded prior to loading and at each time point (Figure 5). Pre-loading peri-implant probing depth were 2.7±0.2 mm for buccal surfaces and 2.6±0.2 mm for lingual surfaces. Similar values were obtained for adjacent periodontal readings prior to loading (2.3±0.13 mm for buccal tooth surfaces and 2.6±0.13 mm for lingual tooth surfaces). There was no statistical significant differences among these initial readings (Figure 5; P>0.05). A slight increase was noted with peri-implant probe depths by 12 weeks compared to before loading values (Figure 5; 3.1±0.2 mm and 3.3±0.4 mm for buccal and lingual implant surfaces, respectively; P>0.05). Adjacent periodontal probing depths remained fairly stable throughout the study evaluation period (2.5±0.3 mm and 2.8±0.1 mm for buccal and lingual tooth surfaces, respectively).

_Gingival Crevicular Fluid (GCF)_
PICF volume was statistically lower than adjacent GCF volume prior to loading (Figure 6; 0.7±0.1 ul and 1.1±0.1 ul, for implant and tooth sites, respectively; P=0.03). As expected with crown delivery, PICF volume significantly increased following loading with each time value being higher than baseline volume (P<0.004). Similarly, PICF volume was statistically higher than GCF volume at each time point following loading (Figure 6; P<0.04).

**Resonance Frequency Analysis**

The mean implant stability quotient (ISQ) prior to loading was 76.5±1.7 (Figure 7). A statistically significant increase in ISQ values was observed at 1 week (79.9±1.5; P<0.001) and at 3, 6 and 12 weeks (Figure 7; 80.67±1.6, 81.5±1.5 and 83.7±1.7, respectively; P<0.0001) compared to baseline (before loading) ISQ values. The differences between post-loading values at each time point were not statistically significant (P>0.05). A mean increase of 7.2 ISQ was observed from baseline to week twelve.

**Crevicular Fluid Content Prior to and Post-loading**

Several cytokines and growth factors (e.g PDGF, IL-4, IL-6, IL-7, IL-10, IL-12, IL-17, Eotaxin, FGFb, IFNγ and TNF-α) were expressed at detectable, but low levels at both peri-implant and adjacent GCF samples, with no differential expression throughout observation period (Table III). IL-1β, IL-1ra, IL-8, G-CSF, IP-10, MCP-1, MIP-1b and VEGF presented higher levels of expression in GCF samples compared to PICF samples.
prior to loading; although there was no statistically significant difference between these baseline values. IL-1β, IL-1ra, IL-8, G-CSF, IP-10, MCP-1, MIP-1b and VEGF were highly expressed within PICF samples following loading and are discussed here.

IL-1β (interleukin-1 beta) is a pro-inflammatory cytokine, thought to work in conjunction with TNF-alpha in bone and soft tissue remodeling. The total amount of IL-1β within PICF prior to loading was 5.4±0.8 pg, lower than GCF IL-1β levels at baseline (11±1.2 pg) (Figure 8). PICF IL-1β total amount was doubled by the first week of loading reaching a total amount of 10.4±2 pg and this amount was similar to adjacent GCF IL-1β levels (10.8±1.5 pg; P>0.05). A decrease in both PICF and GCF IL-1β total amount was noted at 3, 6 and 12 weeks of loading with no statistically significant difference between and within groups (P>0.05).

IL-1ra (interleukin-1ra) is a pro-inflammatory cytokine. The total amount of IL-1ra within PICF prior to loading was 13179±3266 pg which was more than two fold less than baseline GCF levels (28523±7263 pg) (Figure 9). However, the difference between groups was not statistically significant due to large variation observed in data distribution (P>0.05). IL-1ra PICF and GCF levels stayed fairly stable throughout observation period with no statistical significance at different time points (Figure 9).

IL-8 (interleukin-8) is a pro-inflammatory cytokine. It acts as a powerful chemoattractant for neutrophils. PICF IL-8 total amount was 43±10 pg prior to loading, close to a twofold less amount than adjacent GCF levels (78±17 pg; Figure 10). The difference between two groups was not statistically significant (P=0.09). A twofold increase in PICF IL-8 was observed at 1 week post-loading (89±28 pg) compared to GCF
IL-8 total amount at the same time point (53±9 pg). The difference between groups was not statistically significant due to large variation observed at implant sites (P>0.05; Figure 10). GCF and PICF IL-8 levels remained at stable levels after 3 weeks post-loading period.

G-CSF (granulocyte-colony stimulating factor) is a pro-inflammatory cytokine thought to be a major regulator of bone marrow progenitor cells, especially polymorphonucleus leukocytes. [117] Preloading PICF G-CSF total amount was 6±1.3 pg which was lower than baseline GCF levels (10±1.4 pg; P=0.08) (Figure 11). A slight increase in PICF G-CSF presence was noted at week one. A more notable increase was observed at week three, post-loading (10.5±3.7pg). The increase observed at week three was not statistically significant, mainly due to large variations observed between subjects (Figure 11).

IP-10 (interferon-inducible protein-10) is a pro-inflammatory cytokine. Preloading PICF IP-10 total amount was 22±6 pg. It was lower than baseline GCF levels (64±24 pg) (Figure 12; P=0.08). PICF IP-10 levels appeared to increase following three weeks post-loading (2.5 fold increase by week 12; Figure 12). However, standard error values were too high to reach statistically significant results (P>0.05).

MCP-1 (monocyte chemotactic protein-1) is a chemotactic pro-inflammatory cytokine involved in recruitment of macrophages. Preloading PICF MCP-1 total amount was 7±3.4 pg with no statistically significant differences between groups at baseline (Figure 13; P=0.3). PICF MCP-1 levels reached GCF levels by week 1 post-loading and this increase became statistically significant by 12 week post-loading (8.6±2 pg; P=0.01).
MIP-1b (macrophage inflammatory protein-1b) is a chemotactic pro-inflammatory cytokine involved in macrophage recruitment during wound healing. Pre-loading PICF MIP-1b total amount was 7±3.5 pg, no significant difference with GCF baseline levels (Figure 14; P>0.05). There was a 1.7 fold increase in PICF MIP-1b levels by 1 week post-loading (12±4 pg). However, this increase was not statistically significant (P>0.05). The increase in PICF MIP-1b total amount remained elevated through week twelve post-loading. The difference between PICF and GCF MIP-1b content became statistically significant by week 12 (4.4±0.5 pg in GCF and 8.5±2 pg in PICF, respectively; P=0.02).

VEGF (vascular endothelial growth factor) is a pro-inflammatory cytokine involved with the development of capillary formation and permeability during wound healing. The pre-loading difference between GCF and PICF levels of VEGF was not statistically significant (Figure 15; 34±4.5 pg and 22.5±3 pg, respectively; P>0.05). PICF VEGF total amount increased to similar GCF levels at week 1 post-loading and remained stable for the rest of the twelve week observation period for both PICF and GCF levels (Figure 15; P>0.05).

Microbiological Findings

Polymerase Chain Reaction (PCR) assays were conducted to detect the presence of eight putative periodontal pathogens previously reported to be related to peri-implant bacterial flora (Figure 16). V. parvula was detected in about 60 to 70% of implant sites at each time point and about 50 to 70% of adjacent tooth sites at each time point. Similarly,
*S. mitis* was commonly found at implant and tooth sites, 50-70% of implant sites and 50-80% of tooth sites. In addition, *P. micros* and *F. neviforme* were moderately detected at both sites 20 to 60%. *T. forsythia* and *C. gingivalis* were detected at lower percentages, 0 to 40%. Surprisingly, *T. denticola* was detected rarely throughout the study. However, its presence increased with a detection rate of 14% of subjects by week 12. This expression was specific to implant only. (Figure 16)

**Correlations Between Clinical Parameters and Biomolecules**

Correlation analysis among clinical parameters revealed that PI and GI around teeth were highly correlated (P≤0.001; r=0.4). However, similar correlation was not detected between these indices around implants (P=0.58; r=-0.66). *(Table 4)*

When PICF cytokine levels were analyzed for possible correlation, several were found to be highly correlated with each other. *(Table 5)*

Correlation analysis comparing peri-implant clinical parameters with PICF cytokine levels revealed that total amount of five (IL-1beta, IL-8, G-CSF, MIP-1b, and VEGF) out of eight cytokines were statistically correlated with PICF volume. *(Table 6)* Also, GI was statistically correlated with PICF MCP-1 expression (P=0.014, r=0.284). *(Table 6)* Surprisingly, ISQ was significantly correlated with peri-implant PICF G-CSF, IP-10, and MCP-1 total amount. *(Table 6)*
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<th>Appointment</th>
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<td>1</td>
<td>Patient Screening</td>
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<td>2</td>
<td>Comprehensive Examination</td>
</tr>
<tr>
<td>3</td>
<td>One Stage Implant Surgery</td>
</tr>
<tr>
<td></td>
<td><strong>Osseointegration Healing Period</strong></td>
</tr>
<tr>
<td>4</td>
<td><strong>Implant Impression for crown fabrication</strong>&lt;br&gt;• PA with impression post in place&lt;br&gt;• Open tray direct transfer impression</td>
</tr>
</tbody>
</table>
|             | **Base line data collection, crown insertion**
| 1. Gingival Indices  
  a. Plaque Index  
  b. Gingival Inflammation  
  c. Probe Depths  
  d. Attached Gingival Measurements |
| 2. GCF Natural Tooth Sample  
  a. Four 20 second samples from adjacent teeth (two from each tooth) |
| 3. PICF Implant Samples  
  a. Four 20 second samples from the implant (each sample from different sites) |
| 4. Resonance Frequency Analysis (ISQ)  
  a. 4 readings (two buccal two lingual) |
| 5. Samples Stored at -80°C |
| 5. 1 week data collection |
| 1. Gingival Indices  
  a. Plaque Index  
  b. Gingival Inflammation  
  c. Probe Depths |
| 2. GCF Natural Tooth Sample  
  a. Four 20 second samples from adjacent teeth (two from each tooth) |
| 3. PICF Implant Samples  
  a. Four 20 second samples from the implant (each sample from different sites) |
| 4. Resonance Frequency Analysis (ISQ)  
  a. 4 readings (two buccal two lingual) |
| 5. Samples Stored at -80°C |
| 7. 3 week data collection |
| 1. Gingival Indices  
  a. Plaque Index  
  b. Gingival Inflammation  
  c. Probe Depths |
| 2. GCF Natural Tooth Sample  
  a. Four 20 second samples from adjacent teeth (two from each tooth) |
| 3. PICF Implant Samples  
  a. Four 20 second samples from the implant (each sample from different sites) |
Table 1: Continued

<table>
<thead>
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<th>6 week data collection</th>
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<td>a. Plaque Index</td>
</tr>
<tr>
<td></td>
<td>b. Gingival Inflammation</td>
</tr>
<tr>
<td></td>
<td>c. Probe Depths</td>
</tr>
<tr>
<td>2.</td>
<td>GCF Natural Tooth Sample</td>
</tr>
<tr>
<td></td>
<td>a. Four 20 second samples from adjacent teeth (two from each tooth)</td>
</tr>
<tr>
<td>3.</td>
<td>PICF Implant Samples</td>
</tr>
<tr>
<td></td>
<td>a. Four 20 second samples from the implant (each sample from different sites)</td>
</tr>
<tr>
<td>4.</td>
<td>Resonance Frequency Analysis (ISQ)</td>
</tr>
<tr>
<td></td>
<td>a. 4 readings (two buccal two lingual)</td>
</tr>
<tr>
<td>5.</td>
<td>Samples Stored at -80°C</td>
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</table>

<table>
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<td>a. Plaque Index</td>
</tr>
<tr>
<td></td>
<td>b. Gingival Inflammation</td>
</tr>
<tr>
<td></td>
<td>c. Probe Depths</td>
</tr>
<tr>
<td></td>
<td>d. Attached Gingival Measurements</td>
</tr>
<tr>
<td>2.</td>
<td>GCF Natural Tooth Sample</td>
</tr>
<tr>
<td></td>
<td>a. Four 20 second samples from adjacent teeth (two from each tooth)</td>
</tr>
<tr>
<td>3.</td>
<td>PICF Implant Samples</td>
</tr>
<tr>
<td></td>
<td>a. Four 20 second samples from the implant (each sample from different sites)</td>
</tr>
<tr>
<td>4.</td>
<td>Resonance Frequency Analysis (ISQ)</td>
</tr>
<tr>
<td></td>
<td>a. 4 readings (two buccal two lingual)</td>
</tr>
<tr>
<td>5.</td>
<td>Samples Stored at -80°C</td>
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### Table 2: Description Demographics

<table>
<thead>
<tr>
<th>Description Demographics</th>
<th>Data</th>
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<tbody>
<tr>
<td>Total Number Participants In Study</td>
<td>15</td>
</tr>
<tr>
<td>Total Complete</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>Male Participants</td>
<td>8 (53.3%)</td>
</tr>
<tr>
<td>Female Participants</td>
<td>7 (46.7%)</td>
</tr>
<tr>
<td>Average Age of Participant</td>
<td>54 ±3 yrs</td>
</tr>
<tr>
<td>Tooth Type</td>
<td>11 molars / 4 premolar</td>
</tr>
<tr>
<td>Anatomic Location</td>
<td>11 mandible / 4 maxilla</td>
</tr>
<tr>
<td>Osseointegration Healing Time (prior to loading)</td>
<td>6.1 ±0.3 months [4-9 months]</td>
</tr>
<tr>
<td>Implant diameter</td>
<td>4.7 ±0.15 mm [3.7-6 mm]</td>
</tr>
<tr>
<td>Implant length</td>
<td>12 ±0.3 mm [10-13 mm]</td>
</tr>
<tr>
<td>Total Successful</td>
<td>14 (93.3%)</td>
</tr>
<tr>
<td>Total Failed</td>
<td>1 (6.7%)</td>
</tr>
<tr>
<td>Early (pre-loading)</td>
<td>0</td>
</tr>
<tr>
<td>Late (post-loading)</td>
<td>1</td>
</tr>
</tbody>
</table>
### Table 3: Pro-inflammatory Biomarkers Distribution in PICF and GCF

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>pg (+/- se)</th>
<th>Before loading</th>
<th>1 Week</th>
<th>3 Weeks</th>
<th>6 Weeks</th>
<th>12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF</td>
<td></td>
<td>Tooth</td>
<td>Implant</td>
<td>Tooth</td>
<td>Implant</td>
<td>Tooth</td>
</tr>
<tr>
<td>IL-1B</td>
<td>10.95 (1.215)</td>
<td>5.387 (0.7904)</td>
<td>10.8 (1.541)</td>
<td>10.4 (2.088)</td>
<td>8.673 (1.911)</td>
<td>7.713 (1.704)</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>28523 (7263)</td>
<td>13179 (3266)</td>
<td>21522 (7315)</td>
<td>14788 (3137)</td>
<td>15371 (3157)</td>
<td>13109 (3616)</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.1053 (0.01693)</td>
<td>0.08933 (0.01446)</td>
<td>0.1047 (0.01864)</td>
<td>0.1173 (0.02108)</td>
<td>0.1 (0.02021)</td>
<td>0.09267 (0.01867)</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.804 (0.2129)</td>
<td>0.4513 (0.1192)</td>
<td>0.7427 (0.2004)</td>
<td>0.6613 (0.1965)</td>
<td>0.8013 (0.2002)</td>
<td>0.6927 (0.2011)</td>
</tr>
<tr>
<td>IL-7</td>
<td>0.02533 (0.02033)</td>
<td>0.004 (0.004)</td>
<td>0.09267 (0.05943)</td>
<td>0.158 (0.1171)</td>
<td>0.146 (0.09607)</td>
<td>0.112 (0.07232)</td>
</tr>
<tr>
<td>IL-8</td>
<td>77.8 (16.94)</td>
<td>43.46 (10.09)</td>
<td>52.93 (9.311)</td>
<td>89 (28.16)</td>
<td>52.53 (11.84)</td>
<td>47.6 (11.79)</td>
</tr>
<tr>
<td>IL-10</td>
<td>2.473 (0.3668)</td>
<td>1.993 (0.2607)</td>
<td>2.52 (0.4224)</td>
<td>2.443 (0.3302)</td>
<td>2.393 (0.3836)</td>
<td>2.247 (0.305)</td>
</tr>
<tr>
<td>IL-12</td>
<td>3.14 (0.6746)</td>
<td>2.117 (0.3796)</td>
<td>3.443 (0.7256)</td>
<td>2.933 (0.559)</td>
<td>3.227 (0.7435)</td>
<td>2.57 (0.5145)</td>
</tr>
<tr>
<td>IL-17 p</td>
<td>1.74 (0.1656)</td>
<td>1.953 (0.2509)</td>
<td>1.835 (0.2124)</td>
<td>1.88 (0.2614)</td>
<td>1.8 (0.1746)</td>
<td>1.573 (0.1739)</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>0.8807 (0.211)</td>
<td>1.115 (0.2092)</td>
<td>1.027 (0.2448)</td>
<td>1.027 (0.2262)</td>
<td>1.033 (0.2088)</td>
<td>1.147 (0.2136)</td>
</tr>
<tr>
<td>FGFb</td>
<td>2.28 (0.116)</td>
<td>2.26 (0.1767)</td>
<td>2.22 (0.1061)</td>
<td>2.367 (0.3023)</td>
<td>2.207 (0.1217)</td>
<td>2.087 (0.1549)</td>
</tr>
<tr>
<td>GCSF</td>
<td>9.533 (1.43)</td>
<td>5.962 (1.304)</td>
<td>7.967 (1.245)</td>
<td>6.86 (1.297)</td>
<td>9 (1.278)</td>
<td>10.53 (3.677)</td>
</tr>
<tr>
<td>IFNγ</td>
<td>3.72 (0.2509)</td>
<td>2.887 (0.19)</td>
<td>3.967 (0.3718)</td>
<td>3.687 (0.3135)</td>
<td>3.667 (0.3572)</td>
<td>3.167 (0.3418)</td>
</tr>
<tr>
<td>IP10</td>
<td>64.4 (24.49)</td>
<td>21.93 (5.955)</td>
<td>34.05 (10.53)</td>
<td>22.43 (4.776)</td>
<td>32.75 (9.604)</td>
<td>43.87 (16.49)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>10.5 (2.53)</td>
<td>7.167 (1.578)</td>
<td>9.8 (2.377)</td>
<td>9.4 (2.22)</td>
<td>9.313 (2.12)</td>
<td>8.64 (2.054)</td>
</tr>
<tr>
<td>MIP1b</td>
<td>7.62 (3.422)</td>
<td>6.967 (3.459)</td>
<td>4.913 (1.242)</td>
<td>11.77 (3.931)</td>
<td>4.593 (1.094)</td>
<td>8.207 (2.457)</td>
</tr>
<tr>
<td>TNFa</td>
<td>3.1 (0.3179)</td>
<td>1.887 (0.3389)</td>
<td>2.667 (0.3886)</td>
<td>2.493 (0.419)</td>
<td>2.447 (0.3414)</td>
<td>2.933 (0.3283)</td>
</tr>
<tr>
<td>VEGF</td>
<td>33.67 (4.524)</td>
<td>22.47 (3.025)</td>
<td>31.93 (2.891)</td>
<td>30.33 (3.654)</td>
<td>29.4 (3.11)</td>
<td>24.6 (3.016)</td>
</tr>
</tbody>
</table>

Data labeled in Blue is also presented in Figures 8 through 15.
Table 4: Spearman Correlation for Clinical Parameters at Implant and Tooth Sites

<table>
<thead>
<tr>
<th>IMPLANT</th>
<th>PICF</th>
<th>PI</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>P=0.516</td>
<td>r=-0.077</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>P=0.979</td>
<td>r=0.003</td>
<td>P=0.575</td>
</tr>
<tr>
<td>RFA</td>
<td>P=0.08</td>
<td>r=0.207</td>
<td>P=0.623</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TOOTH</th>
<th>GCF</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCF</td>
<td>P=0.557</td>
<td>r=0.069</td>
</tr>
<tr>
<td>PI</td>
<td>P=0.557</td>
<td>r=0.069</td>
</tr>
<tr>
<td>GI</td>
<td>P=0.490</td>
<td>r=0.082</td>
</tr>
</tbody>
</table>

Table 5: Spearman Correlation Analysis for Cytokine Data at Implant Sites

<table>
<thead>
<tr>
<th></th>
<th>IL-1β</th>
<th>IL-1ra</th>
<th>IL-8</th>
<th>G-CSF</th>
<th>IP-10</th>
<th>MCP-1</th>
<th>MIP-1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>P≤0.001</td>
<td>r=0.506</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1ra</td>
<td>P≤0.001</td>
<td>r=0.591</td>
<td>P≤0.001</td>
<td>r=0.525</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>P≤0.001</td>
<td>r=0.645</td>
<td>P≤0.001</td>
<td>r=0.591</td>
<td>P≤0.001</td>
<td>r=0.651</td>
<td></td>
</tr>
<tr>
<td>G-CSF</td>
<td>P≤0.001</td>
<td>r=0.014</td>
<td>P=0.001</td>
<td>r=0.301</td>
<td>P≤0.001</td>
<td>r=0.583</td>
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</tr>
<tr>
<td>IP-10</td>
<td>P=0.014</td>
<td>r=0.214</td>
<td>P=0.001</td>
<td>r=0.370</td>
<td>P≤0.001</td>
<td>r=0.651</td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>P=0.005</td>
<td>r=0.325</td>
<td>P=0.04</td>
<td>r=0.242</td>
<td>P≤0.001</td>
<td>r=0.719</td>
<td>P=0.006</td>
</tr>
<tr>
<td>MIP-1b</td>
<td>P=0.005</td>
<td>r=0.323</td>
<td>P=0.001</td>
<td>r=0.721</td>
<td>P≤0.001</td>
<td>r=0.485</td>
<td>P≤0.001</td>
</tr>
<tr>
<td>VEGF</td>
<td>P≤0.001</td>
<td>r=0.610</td>
<td>P≤0.001</td>
<td>r=0.584</td>
<td>P≤0.001</td>
<td>r=0.735</td>
<td>P=0.385</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>P≤0.001</td>
<td>r=0.448</td>
<td>P≤0.001</td>
<td>r=0.436</td>
<td>P≤0.001</td>
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</table>
Table 6: Spearman Correlation between Clinical Parameters and Cytokines at Implant Sites

<table>
<thead>
<tr>
<th></th>
<th>PICF</th>
<th>PI</th>
<th>GI</th>
<th>ISQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>P=0.004</td>
<td>r=0.332</td>
<td>P=0.225</td>
<td>r=0.143</td>
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<tr>
<td>IL-1ra</td>
<td>P=0.689</td>
<td>r=0.047</td>
<td>P=0.888</td>
<td>r=0.017</td>
</tr>
<tr>
<td>IL-8</td>
<td>P=0.021</td>
<td>r=0.269</td>
<td>P=0.602</td>
<td>r=0.062</td>
</tr>
<tr>
<td>G-CSF</td>
<td>P=0.02</td>
<td>r=0.271</td>
<td>P=0.591</td>
<td>r=-0.064</td>
</tr>
<tr>
<td>IP-10</td>
<td>P=0.190</td>
<td>r=0.155</td>
<td>P=0.298</td>
<td>r=-0.123</td>
</tr>
<tr>
<td>MCP-1</td>
<td>P=0.304</td>
<td>r=0.121</td>
<td>P=0.944</td>
<td>r=0.008</td>
</tr>
<tr>
<td>MIP-1b</td>
<td>P≤0.001</td>
<td>r=0.416</td>
<td>P=0.468</td>
<td>r=-0.086</td>
</tr>
<tr>
<td>VEGF</td>
<td>P=0.022</td>
<td>r=0.265</td>
<td>P=0.611</td>
<td>r=0.06</td>
</tr>
<tr>
<td>Pro-inflammatory Mediator</td>
<td>Sources</td>
<td>Target/Action</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------</td>
<td>---------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGF (Platelet Derived Growth Factor)</td>
<td>Platelets – active</td>
<td>Activate platelet cells, aiding in the inflammatory and clot formation process</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1 Beta (Interleukin 1 Beta)</td>
<td>Monocyte, Macrophage, T and B Cells, Endothelial cells, Fibroblasts, Smooth Muscle</td>
<td>T and B cells, Monocytes, Fever, acute phase response, neutrophil production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-CSF (Granulocyte Colony Stimulating Factor)</td>
<td>Endothelium and macrophages</td>
<td>Proliferation and differentiation of granulocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIP-1b (Macrophage Inflammatory Factor 1)</td>
<td>Macrophages</td>
<td>Chemokine that leads to migration of lymphocytes and monocytes during wound healing, early implant mucositis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1 (Monocyte Chemotactic Protein – 1)</td>
<td>Monocytes, Endothelial cells</td>
<td>Chemotactic for Monocytes not neutrophils Basophil activator and degranulation induction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF (Vascular Endothelial Factor)</td>
<td>Endothelial cells</td>
<td>Promote capillary formation and dilation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP-10 (Interferon-inducible Protein – 10)</td>
<td>Neutrophils stimulated by INF-gamma, TNF-alpha, or IL-1 beta</td>
<td>Activator and attractant of T cells May play a role in hypersensitivity reactions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1 ra (Interleukin 1 receptor antagonist)</td>
<td>Monocytes, macrophages, neutrophils fibroblasts, others</td>
<td>Competitively inhibits IL-1a and IL-1b, does not elicit same response as IL-1a/b, role in inflammation unclear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4 (Interleukin 4)</td>
<td>Mast cells, T cells, Bone Marrow Cells</td>
<td>Growth factor for hematopoietic cells and lymphocytes. Stimulates colony formation of neutrophils, eosinophils, basophils, erythroid, megakaryocyte, and monocyteic cells. Not lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (Interleukin 6)</td>
<td>T and B cells, macrophages, bone marrow stromal cells, fibroblasts, endothelial cells</td>
<td>Regulates B and T cell functions, Induces acute phase response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-7 (Interleukin 7)</td>
<td>Bone marrow stromal cells, thymic stromal cells, spleen cells</td>
<td>Growth factor for B and T cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8 (Interleukin 8)</td>
<td>Monocytes, Lymphocytes, Granulocytes, Fibroblasts, Endothelial cells, and more</td>
<td>Chemokine. Primary action is to attract and activate neutrophils, angiogenic activity, attracts some lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10 (Interleukin 10)</td>
<td>Activated CD4 and CD8 T cells</td>
<td>Proliferation of B cells, thymocytes, and mast cells, Stimulates IgA fabrication of B cells, suppresses Th1 cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-12 (Interleukin 12)</td>
<td>Macrophages and dendritic cells</td>
<td>Differentiation of TH1 T Helper cells, induces INF-γ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-17 (Interleukin 17)</td>
<td>CD4 T cells</td>
<td>Hematopoiesis by inducing cytokine production by epithelial cells, fibroblast, endothelial, and stromal cells. Enhances ICAM-1 making cells more adherent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INF-γ</td>
<td>CD4 and CD8</td>
<td>Activation, growth and differentiation of T and B cells, macrophages, Uregulates MHC expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-alpha (Tumor Necrosis Factor – Alpha)</td>
<td>Monocytes, macrophages, other</td>
<td>Production of adhesion molecules, chemokine, and cytokine inducing IL-1, IL-6, IL-8, and MMPs. In conjunction with IL-1 induces osteoclast activation,</td>
<td></td>
<td></td>
</tr>
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Figure 1: Surgical Guide on Diagnostic Cast

1.1
Buccal view of surgical guide identifying implant angulation and proposed cervical location of implant crown.

1.2
Occlusal view of a surgical guide with a lingual access lot on the diagnostic cast.

1.3
Occlusal view of a surgical guide with a pilot hole on the diagnostic cast.
Figure 2: Plaque Accumulation Prior To and Post Crown Delivery

Bl: Before Loading; data is presented as mean Plaque Index ± standard error
*P<0.004 Difference between implant and teeth at each week
**P=0.02 Difference at baseline (before loading) between implant and tooth
Figure 3: Soft Tissue Characteristics Prior to and Post Crown Delivery

Data is expressed as mean GI ± standard error

*P<0.05 comparison of implant GI with teeth GI at BL and 1 week

**P<0.001 Comparison of baseline implant GI with week 12 implant GI
Figure 4: The amount of Keratinized Tissue prior to and post loading

Keratinized Tissue

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mm (mean±se)
Figure 5: Changes in peri-implant and adjacent periodontal probing depths prior to and following loading
Figure 6: Peri-implant and Periodontal Crevicular Fluid Volume Changes prior to and following loading

CREVICULAR FLUID VOLUME

*P=0.03 baseline PICF and GCF
**P<0.004 PICF – difference between BL and WK 1, 3, 6, 12
***P<0.04 PICF with GCF each time point
Data is presented as total amount of protein obtained within 20 second crevicular fluid sampling.
Figure 7: Resonance Frequency Analysis prior to and following loading

![Bar chart showing Resonance Frequency Analysis](image)

**P<0.01 compared to baseline
***P<0.0001 compared to baseline

Data is presented as total amount of protein obtained within 20 second crevicular fluid sampling.
Figure 8: PICF and GCF IL-1β total amount prior to and following loading

P>0.05 between and within groups
Data is presented as total amount of protein obtained within 20 second crevicular fluid sampling.
Figure 9: PICF and GCF IL-1ra Total Amount Prior to and Following Loading

P>0.05 between and within group differences

Data is presented as total amount of protein obtained within 20 second crevicular fluid sampling.
Figure 10: PICF and GCF IL-8 Total Amount Prior to and Following Loading

P > 0.05 between and within groups
Data is presented as total amount of protein obtained within 20 second crevicular fluid sampling.
Figure 11: PICF and GCF G-CSF Total Amount Prior to and Following Loading

Data is presented as total amount of protein obtained within 20 second crevicular fluid sampling.

P>0.05 between and within groups differences
Figure 12: PICF and GCF IP-10 Total Amount Prior to and Following Loading

P > 0.05 between and within group differences

Data is presented as total amount of protein obtained within 20 second crevicular fluid sampling.
Figure 13: PICF and GCF MCP-1 Total Amount Prior to and Following Loading

*P=0.014 PICF levels at 12 weeks compared to PICF levels at baseline
Data is presented as total amount of protein obtained within 20 second crevicular fluid sampling.
Figure 14: PICF and GCF MIP-1b Total Amount Prior to and Following Loading

*M<0.02 PICF compared to GCF detection at week 12

Data is presented as total amount of protein obtained within 20 second crevicular fluid sampling.
Figure 15: PICF and GCF VEGF Total Amount Prior to and Following Loading

Data is presented as total amount of protein obtained within 20 second crevicular fluid sampling.

P>0.05 between and within group differences
Figure 16: Microflora Formation – Implant and Tooth

**FLORA FORMATION-IMPLANT**

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**FLORA FORMATION-TOOTH**

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CHAPTER IV: DISCUSSION

The physiological healing response to implant placement surgery is an acute and well controlled activation of several pro-inflammatory pathways affecting both hard and soft tissue components of the wound. This short term response may become reactivated following occlusal loading of the implant through remodeling procedure. [10] [12] [116] [117] [118] The ultimate goal of this study was to detect peri-implant tissue changes that occur due to bone and soft tissue remodeling as a response to mechanical loading. Specifically, an investigation on clinical parameters and the expression of local cytokines and growth factors within peri-implant crevicular fluid and adjacent gingival crevicular fluid was conducted in order to differentiate peri-implant soft tissue response prior to and following occlusal loading. To our knowledge this is the first patient based study evaluating early peri-implant soft tissue responses immediately following mechanical loading following a conventional implant protocol.

Study population included 15 non-smokers with similar gender and age distribution. None of the edentulous sites was previously grafted. It has been reported that peri-implant wound healing is complicated in smokers. [119] [120] Many studies report that smokers may experience higher rates of implant failure. [121] [122] [123] [124] Thus, smokers were excluded from this study. Although controversial, some studies report differential responses in peri-implant clinical parameters, implant stability,
and long term survival rate for implants placed at grafted sites. [125] Thus, grafted sites were excluded from the study. Older age may affect wound healing rate in general. [126] [127] Most of the patients in need of implant placement are older individuals. Study design recruited patients as old as 75 yrs. Efforts were made to have an equal presentation of adult age groups. Gender differences have not been shown as a possible risk indicator for implant failure. [128] [129] [130] Gender distribution within this study was similar and should not have any effect on results. Patients with a history of parafunctional habits were also excluded since this is a well-known risk factor for dental implant failure. [131] [132] [133] All implants were placed according to a one-stage surgical protocol to help control the amount of possible scar formation at soft tissue level. In addition, all implant restorations were screw retained to allow evaluation of implant stability with ISQ at post loading evaluation visits. Most of the implants were placed in mandibular posterior sextant. All implants included in the study presented with baseline ISQ values >50 which allowed for one-stage surgical protocol. There was one implant failure observed at the twelfth week evaluation appointment. It was localized in a maxillary posterior sextant which is documented to possess a higher risk of failure. [134] [135] [136] [137] 

The average osseointegration period prior to loading was 6.1±0.3 months with a range of four to nine months in the current study. It is possible that the variation in osseointegration healing times may have affected the results of the study. There is controversial data on how peri-implant tissues response to long-term healing period in the absence of occlusal forces. According to the mechanostat theory of bone remodeling, developed by Frost, [26] one might speculate bone atrophy may occur around an implant
if the healing period persists long enough without mechanical stimulus to the implant-bone interface. Romanos’ [138] observations of unloaded implant at three months exhibiting less dense peri-implant bone with increased adipose tissue compared to a loaded implant, supports this theory. However, Mattieu et al [139] reported, using a rabbit model, increased bone to implant contact of unloaded implants at fourteen weeks post surgical placement when compared to seven weeks, 69% and 27% respectively. Similarly, Carr et al [140] observed, using a baboon model, an increase in bone to implant contact at six months when compared to three months, 49.2% and 38.8% respectively. There is sufficient data to suggest healing periods longer than conventional three months in the mandible and six months for maxilla without occlusal loading does not affect implant success and/or survival rate. In addition, though not a direct occlusal function, implants placed with one stage protocol experience some mechanical loading since they are exposed to masticatory forces as well as forces applied by muscles such as cheek and tongue. [117]

Clinical evaluation of the soft tissue response to occlusal loading included: plaque accumulation, gingival inflammation, changes in probing depth, and keratinized tissue width. In general, all plaque scores were exhibited low levels for both implant and adjacent teeth sites. Peri-implant surfaces observed significantly lower plaque accumulation than teeth which is similar to previous reports for plaque accumulation around implant supported restorations. [141] [142] [143] Lower plaque scores around implants have been attributed to the restorative material used. All restorations were metal ceramic restoration with no metal showing in the current study. Dental porcelains have
been reported to minimize plaque formation as a result of their highly polished surface and low surface energy. [144][145][146][147]

Peri-implant gingival inflammation, recorded as modified gingival index (GI) score, exhibited an increase following loading in the current study. This is despite to low PI scores. There was an inverse correlation of implant PI and implant GI scores in the data. Studies investigating peri-implant gingival tissue characteristics generally report similar or slightly higher GI scores around healthy implants compared to healthy teeth. [142][148][149] Gingival inflammation is generally directly correlated to plaque accumulation. It appears that differential GI response detected in the current study prior to and following loading may be directly related to both crown insertion and functional loading.

Peri-implant and adjacent periodontal probing depths were used to evaluate soft tissue response to implant loading. A slight increase was observed in peri-implant probe depths by week twelve after loading compared to pre-loading values. This agrees with previously reported literature. [146][149] Differences in soft tissue attachment around dental implants compared to gingival attachment around a tooth result in an increased probability that the tip of a periodontal probe will pass through the junctional epithelium and implant-gingival attachment even in health. [50][83] This will result in deeper peri-implant probe depth measurements. An additional compounding factor is the potential size discrepancy of a full contour implant restoration emerging from a significantly smaller implant fixture. The size discrepancy results in difficulty with probing peri-implant sulci and may result in iatrogenic damage to the peri-implant
gingival tissue. Most crowns inserted were greater diameter than the implant and implant healing abutment. The insertion of an implant crown resulted in expansion and pressure on the peri-implant gingival tissues. However, there was no implant surface exposure and/or gingival recession.

The results of the study suggest during the twelve week evaluation period following implant loading little to no changes to keratinized gingival width should be expected. Similar information evaluating the effects of loading on peri-implant keratinized gingiva immediately following loading is lacking in the literature. However, the necessity of keratinized tissue around implants for long-term success has been discussed in depth. Most studies suggest peri-implant keratinized tissue is necessary to maintain long-term peri-implant health. In contrast others report that keratinized gingival width does not affect the risk of gingival recession or peri-implant gingival health. The lack of keratinized tissue was an exclusion criterion in the current study. Thus, it was not possible to compare peri-implant clinical parameters in the absence of keratinized tissue.

Resonance Frequency Analysis (RFA) was used to determine implant stability in the current study. A statistically significant increase in ISQ levels was noted following implant loading. Literature on ISQ levels at early phases of post loading is very limited. Most of the available studies compare ISQ values of immediately loaded implants with implants following a conventional osseointegration period. These studies report similar ISQ values following implant surgery. Several in vitro and in vivo studies suggest that implants effectively transfer functional stress to the bone via the
intimate bone to implant interface. [18] [35] [34] Thus, the theory of “Wolff’s Law” of bone remodeling can be expected to occur in peri-implant bone which is subject to strains produced by occlusal load. [22] [24] [25] [26] Fischer et al. [160] reports ISQ values of immediately loaded implants after twelve months in function with a slight increase of 3.5 ISQ units from baseline surgery. Grunco et al. [161] reports no change in RFA from baseline after to twelve months of service with conventionally loaded implants and a slight increase from baseline to twelve months of 2.3 ISQ with immediately loaded implants. Our data is the first available data in which implant stability has been measured immediately following conventional implant loading. It is reasonable to speculate that the significant increase in RFA values observed in our study is most likely the result of the bone remodeling process that occurs due to physiologic strain in the peri-implant bone via the dental implant-restoration system.

The reason dental literature may lack data regarding implant stability following crown insertion is likely related to the potential risks involved with regaining access to an implant for RFA measurements once the final restoration has been delivered. Potential risks may arise related to the restoration retaining screw, crestal bone loss, and irretrievability. It is documented in the literature that multiple insertions and removals of titanium retaining screws may be detrimental to a screws implant pre-load. [162] [163] [164] Changes in screw preload may increase the risk of screw loosening or breakage. This concern was addressed by not torquing the retaining screws to the recommended final torque values until the final appointment in the current study. The crowns were removed and re-inserted only four times. It has been recommended that screws can be
torque to the manufactures recommendations ten times prior to replacement. None of the subjects had screw loosening or screw breakage during the 12 weeks observation period.

There is also concern that repeated removal and insertion of transmucosal crowns/abutments may induce crestal bone changes. Reports from Grandi [165] and Canullo [166] suggest multiple insertions and removal of an implant abutment is associated with increased crestal bone loss adjacent to an implant. However, these studies report statistically significant differences in crestal bone loss observed with repeated abutment removal; the crestal bone loss reported was 0.1 to 0.2 mm and may not be clinically significant to the long term success of an implant. The crestal bone loss reported with multiple abutment removal and insertions is within the generally accepted implant success criteria presented by Smith and Zarb. [5] There is no evidence to suggest the insertion and removal of the implant crowns as performed in our study will affect the peri-implant soft tissue and characteristics. Peri-implant bone loss/crestal changes could not be evaluated in this study since 12 weeks is too early to differentiate changes in bone level.

A significant increase in peri-implant crevicular (PICF) volume was observed in response to occlusal loading in the current study. Tozum et al. [167] compared PICF to GCF volumes and reports PICF volume to be greater than GCF volume in patients with gingival health. Our results support this observation. However, Murata et al. [99] report PICF volume to be less than GCF volumes in healthy subjects and Apse et al. [93] report no difference. Other studies report conflicting data regarding PICF volumes over time.
with loaded implants. Tozum et al. [168] reports PICF volume fluctuates over a one year period of time with a slight decrease in volume at twelve months of loading. Similarly, Guncu et al. [161] reports a trend of PICF volume decreasing over time. Both these studies report observations after months of loading. Beck et al. [96] describes a maximal increase in PICF volume at the fourth day immediately following crown insertion, but reports a subsequently decrease in volume. In addition Last et al. [97] suggests PICF volumes increase after restoration of an implant and remain elevated even with a lack of visible inflammation. The current study compares baseline PICF to shorter time intervals post loading. Results show an increase in PICF following crown insertion which then stayed similar to adjacent GCF volume throughout observation time. In that aspect, what is reported as post-loading data is similar to most of the published work.

Several factors influence PICF volume. PICF volume is affected by: implant location [95] [98], implant diameter [95], gingival pocket depth [77] [95] [169], plaque presence [100], implant loading [96] [97], and inflammation [77] [82] [95] [98] [99] [169]. The most significant and well documented factor is the presence of gingival inflammation. [77] The second most influential factor may be gingival pocket depth or volume. Larger teeth and larger pockets possess a larger area for fluid collection within the gingival sulcus. Thus, GCF volume increases would be expected. Increases in PICF volumes observed in our study are most likely a result of several factors. Initial PICF volumes obtained were the smallest volumes recorded. This volume obtained is related to the tissue height and healing abutment emergence shape. [77] Inherently PICF volumes obtained around an implant healing abutment at baseline would be less than
PICF volume obtained after crown insertion. Another possible contributing factor to the increase in PICF volume observed, especially at the week one follow-up appointment may be related to the previously mentioned process of gingival tissue remodeling and related inflammatory process.

PICF and adjacent GCF content were investigated prior to and following loading in the current study. Data on IL-1β, IL-1ra, IL-8, G-CSF, IP-10, MCP-1, MIP-1b, and VEGF were presented and discussed here due to their consistent detection levels among various samples as well as their the differential expression between PICF and GCF at different time points.

Pro-inflammatory cytokine detection in peri-implant gingival crevicular fluid has been studied and reported in the dental literature. [92] [98] [100] [101] [102] [103] [105] [104] [107] Most studies discuss changes that may exist related to peri-implant tissue in health and disease [92] [98] [100] [103] [104] [105] [107] or compared to healthy teeth [101] [102], or surgical implant placement wound healing (Emecen-Huja, MS Thesis 2011). Only a limited number of studies have reported on changes in PICF pro-inflammatory cytokines solely with respect to loading. To our knowledge, this study is the first report on pro-inflammatory cytokine detection in PICF samples obtained immediately following implant loading.

Pro-inflammatory cytokines IL-1β, IL-8, MCP-1, MIP-1b and VEGF all demonstrated a detectable PICF increase the first week after loading when compared to baseline PICF levels. None of the increases were statistically significant. Subsequently, the detectable levels of each cytokine or growth factor decreased at week three and
remained at a similar level throughout the evaluation visits. Therefore, it is probable that the increase in IL-1β, IL-8, MCP-1, MIP-1b and VEGF detected at week one following loading was influenced by the simultaneous PICF volume increase, but the trend of subsequent decrease in detectable levels during the following weeks suggests other factors were involved. The increases observed at week one were likely in response to the change in peri-implant environment as a result of the crown insertion and in response to implant loading. These pro-inflammatory cytokines provide evidence for early peri-implant soft tissue response to implant crown insertion and functional loading.

Difference between PICF and GCF levels of IL-1β and VEGF were detected at baseline. IL-1β has been studied extensively in the dental literature. VEGF data is limited. IL-1β is thought to play a significant role in periodontal inflammation and disease as many have demonstrated a detectable increase in IL-1β in inflamed or disease PICF. [95] [98] [100] [103] [104] [105] IL-1β presence in healthy PICF is similar to detectable levels observed in healthy GCF. [99] [102] Our data support this finding once the implant was loaded. Panagakos et al. [105] report IL-1β’s detectable presence of 59.47 pg/site in healthy PICF. Their samples were obtained using four paper strips at thirty seconds each; slightly longer than our twenty second collection times. Our data reports lower total presence in healthy PICF when compared to Panagokos. The significant difference in IL-1β in PICF and GCF recorded at baseline prior to loading appears to be a unique finding; however, it does correlate to previous data reported by our group (Emecen-Huja, MS Thesis 2011) regarding the twelve weeks period wound healing after implant surgery. This data, regarding IL-1β and VEGF requires further investigation
to possibly understand the significance of their elevated levels prior to implant loading when compared to natural teeth.

PICF G-CSF level at week three exhibited a notable, but not statistically significant, increase. Subsequently, at weeks six and twelve the detectable mean levels were returned to similar baseline and one week levels. The dental implant literature is void of references to G-CSF in relation to peri-implant soft tissue. G-CSF is known to be responsible for regulating bone marrow progenitor cells; especially neutrophil progenitor cells. [172] [173] [174] [175] It has also been shown to be involved in angiogenesis. [176] [177] Deficiency in G-CSF has a strong correlation to neutropenic disorders. It has also been used to help successfully treat congenitally neutropenic people. [117]

Recombinant GCSF has been shown to help improve oral health as conjunctive treatment with periodontal maintenance in patients with cyclic neutropenia. [176] [179] In our data, G-CSF is detectable in PICF and appears to increase at week three after loading. This increase does not correlate with PICF volume increases. It does have a significant correlation with RFA data. G-CSF is likely expressed as a response to implant loading and requires further investigation.

Mean levels of IP-10 detected in the patient’s PICF also exhibited a change following loading. At week three mean IP-10 levels increased and continued to increase through week twelve. Comparative data from the literature is limited. Therefore, our data cannot be compared or confirmed with respect to PICF. More research is needed. IP-1 does not correlate with PICF volumes in our data. It does correlate with RFA. It is likely the differential detection of IP-10 in PICF is affected by implant loading.
Mean MIP-1b exhibits a similar pattern as IL-1ra, IL-8, IL-1β, VEGF, and MCP-1. However, this particular pro-inflammatory cytokine at baseline PICF is recorded to be at a similar level as found in GCF at baseline. At week one of loading an increase in mean MIP-1b detection occurs in PICF but not in the GCF. The difference in MIP-1b levels recorded in the PICF and GCF was not statistically significant at week one but became significant at week twelve. Similar to IP-10 and G-CSF, MIP-1b is not well documented in the dental PICF literature. Thus, comparisons are not possible at this time. MIP-1b is reported as a chemokine for monocytes and lymphocytes and is thought to stimulate and help regulate hematopoietic cell production during inflammatory states. [180] [181]

There was significant correlation with detectible cytokines in PICF in relation to each other. This is to be expected. Interestingly, there was significant correlation between G-CSF, IP-10, and MCP-1 with the clinical parameter RFA. IL-1β, IL-8, G-CSF, MIP-1b, and VEGF exhibited a significant correlation with PICF. The correlation does not mean there is a direct cause and effect relationship. However, it does suggest there is a potential relationship with respect to the clinical data we recorded and implant loading, and requires additional investigation.

It is reasonable to speculate that sulcus flora would be affected by the insertion of an implant supported crown. Thus, PCR assays were used to detect the presence of eight specific putative periodontal pathogens.

The change in the peri-implant environment may affect the peri-implant microflora which in turn may additionally affect the peri-implant soft tissue response.
Our data reports the percentage of patients with detectable specific bacteria in PICF and GCF. Evidence suggests microbial colonization of implants occurs early after insertion. Early peri-implant microbial studies suggest healthy implant microbial composition as similar to healthy natural teeth consisting mainly of cocci and non-motile rods. The prevalence of more anaerobic and motile bacteria appears to occur with increased time in the mouth, increased probe depths, and microbial content of adjacent natural teeth. The presence of known pathogenic bacteria has been reported to be present in both healthy and diseased implant tissues. Kumar et al., in a recent study suggests that despite the similarities between peri-implant and periodontal microflora, peri-implant gingival microflora show specific differences. The putative pathogens studied in the current investigation were based on peri-implant microflora characteristics recently reported by Kumar et al. Based on limited amount of samples, it appears that loading did not have a significant effect on peri-implant sulcus flora. However, a potent periodontal pathogen, Treponema denticola was highly expressed within peri-implant sulcus at 12 weeks compared to adjacent gingival sulcus. Casado reports the presence of T. denticola on both healthy and diseased implant surfaces. Conversely, Lee reports T. denticola is not present on healthy implants. Our data may be unique in that T. denticola was detected in few patients only during the twelve week and on implants.

T. denticola, a spirochete bacterium, is considered to be one of the three “Red Complex” periodontal pathogens. The “Red Complex” is composed to P. gingivalis, T.
forsythia, and T. denticola. It is usually present with P. gingivalis due to its bonding affinity with P. gingivalis. When P. gingivalis and T. denticola are present together a significant increase in periodontal destruction occurs. [180]

One of the limitations of this study is small size of study population. The size of N=15 was initially chosen by performing a priori sample size determination test. RFA was chosen as main clinical parameter. Previous studies were reported a 4-5 ISQ unit difference as statistically significant difference at P≤0.05. Ideally, a larger study population may help better interpret local cytokine levels.

Conclusions

In summary the following changes were observed with peri-implant parameters after implant loading:

- Peri-implant plaque accumulation was lower than adjacent teeth throughout the twelve weeks evaluation period.
- Gingival inflammation increased around implants after loading reaching similar levels as the adjacent natural teeth. Peri-implant gingival inflammation was not correlated to peri-implant plaque scores.
- Peri-implant keratinized gingiva width remained unchanged.
- Peri-implant probe depths increased slightly after loading. They were slightly deeper than adjacent teeth by twelve weeks of loading.
- PICF volume experienced a significant increase after loading and remained elevated through week twelve.
• RFA (ISQ) measurements increased significantly from baseline following implant loading throughout the twelve week observation period.

• Cytokines and growth factors such as: PDGF, IL-4, IL-6, IL-7, IL-10, IL-12, IL-17, Eotaxin, FGFb, IFNγ and TNF-α were all detectable in PICF but at low levels and no differential expression throughout the study.

• IL-1β, IL-1ra, IL-8, G-CSF, IP-10, MCP-1, MIP-1b and VEGF exhibited detectable changes in PICF in response to loading.

• Significant individual variations and fluctuations throughout observation time were noticed in various PICF cytokine/growth factor levels.

• There was no difference between pre-loading and post-loading peri-implant sulcus flora members within the limits of specific bacteria. Also, peri-implant sulcus flora was similar to adjacent teeth sulcus flora of the eight species studied.

Our hypothesis states that dental implant loading following conventional osseointegration period affects implant stability, crevicular fluid volume and content. The data from our study supports this hypothesis. Dental loading following conventional osseointegration effects implant stability, PICF, and PICF content.

In response to our specific aims:
1. Implant loading effects peri-implant plaque levels, gingival inflammation, and probe depths.

2. Implant loading significantly affects PICF volume.

3. Implant loading significantly affects implant stability as measured by RFA.
4. Implant loading appears to influence selective inflammatory cytokines and growth factors detectable in PICF.

**Future Direction**

Future direction may include further investigation of soft tissue response to different types of restorations, different restorative materials, different abutment types, evaluation of different molecules present in PICF, and more in depth investigation of peri-implant microflora.
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