SITE-SPECIFIC CHARACTERISTICS OF PERI-IMPLANT WOUND

THESIS

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ABSTRACT

Objective: One-stage implant placement allows evaluation of early healing. This study aims to determine site-specific characteristics of peri-implant wounds.

Material and Methods: Bone level implants in non-smoker patients were simultaneously placed at two posterior quadrants. Resonance Frequency Analysis (RFA) was performed at placement and 4 weeks post-operatively. Bone plate and flap thicknesses were measured with a caliper. Plaque and gingival indices (PI and GI) and probing depth (PD) were determined at baseline and during healing. Crevicular fluid (CF) was collected using sterile strips. Gingival tissue biopsies were obtained for future studies. CF was processed through multiplex assays for several cytokines associated with wound healing. Repeated Measures ANOVA followed by Bonferroni adjustment and Spearman Correlation Coefficient were conducted.

Results: Of twenty-seven subjects that were recruited, twenty-two patients (13 female; 55±3yrs) completed the study. Surgical site A and B were assigned at the first post-operative appointment. Initial bone and flap thicknesses were similar between surgical sites (P>0.05). Probing depth ranged 2.5 to 3.2 mm, with statistically deeper peri-implant PD than periodontal PD at both sites during 4th week (P≤0.03). RFA revealed an initial
69±1.3 ISQ for site A, compared to 73±1.14 ISQ for site B (P=0.03), which changed to 71±1.4 and 69±2.4 ISQ at 4 weeks, respectively. There was no statistically significant difference between baseline and 4 weeks at both sites, and between sites at 4 weeks (P>0.05). PI and GI decreased at both sites by 4th week (P>0.05). Initial CF volume increase at week 1 diminished to baseline levels by week 4, with significant difference within site A at week 1 and 4 (P=0.002), and between site A and B for peri-implant CF at week 1 (P=0.05). Statistically significant differences were detected for IL-1ra expression within site A between gingival (GCF) and peri-implant crevicular fluid (PICF) levels at 1 week (P=0.002) and 4 weeks (P=0.004), and within site B between GCF and PICF levels at 4 week (P=0.002). IL-6 expression also showed significant differences within site A at GCF level between 1 and 4 weeks (P=0.002), and within site B between GCF and PICF levels at 1 week (P=0.02). Lastly, MIP-1β expression between GCF and PICF levels at week 1 (P=0.02), and within PICF level between 1 and 4 weeks showed statistical significance (P=0.03) within site A.

Conclusion: It is possible to detect differential response to surgical trauma at two simultaneously created wound sites within an oral cavity. IL-8, IL-1β, MIP-1β, IL-6 and TNF-α are promising markers to monitor wound healing response in peri-implant tissues. Statistically significant correlations were noted between IP-10 and PICF (P=0.02; r=0.505) and between IL-1β and PICF (P=0.04; r=0.458) after one week of healing. A significant correlation between TNF-α and PD was noted at week 4 (P=0.026; r=0.405)

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DEDICATED TO MY MOM

TERRI S. KIM
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CHAPTER I

INTRODUCTION

Dental implants are currently considered as standard of care for replacement of missing teeth. Although long-term success of implant supported dental restorations is similar to those of conventional dental restorations, the prevalence/incidence of early complications that occur in relation to peri-implant wound healing are not well known. A wound involving both hard and soft tissue components has to be created in order to place a dental implant device into jaw, which causes acute trauma and activates related pro-inflammatory healing pathway. The host response to the surgical trauma has to be well controlled in order to prevent any complications. Currently available tools to evaluate the healing outcome are limited to clinical parameters and they lack both specificity and sensitivity in detecting early complications. Our research team previously reported that acute response may be detected through crevicular fluid analysis, where differential expression of selected cytokines are noted between implant sites and surgically manipulated adjacent tooth sites.¹ The purpose of this current study was to determine whether a site-specific differential response to simultaneously created surgical traumas at two different quadrants within the same oral cavity is detectable. The ultimate goal was to determine the possible variations in peri-implant wound healing that may occur independent from surgeon's experience and implant design.
Wound healing phases

The clinical model used by this study allows evaluation of early phases of peri-implant wound healing at soft tissue levels. Nevertheless, it may be possible to detect some of the bone cytokines induced following osteotomy, at least until epithelial seal establishment.

Soft tissue healing after mucoperiosteal flap elevation and re-adaptation occurs in three distinct phases: (1) inflammation; (2) granulation tissue formation; and (3) matrix formation and remodeling. Upon wound closure, a blood clot fills the wound space. Within one hour, neutrophils infiltrate the clot. Within three days, granulation tissue forms. Neutrophil infiltrate gradually decreases while macrophages increase. By seven days, cell-rich granulation tissue with fibroblasts and young collagen fibers dominates in the wound site, undergoing maturation and remodeling. Tensile strength of the periodontal surgical wounds return to pre-operative levels after 14 days.

The peri-implant soft tissue forms a seal, functioning as a barrier. Connective tissue, a fibrous layer of interwoven fiber bundles, attaches firmly to the implant. Junctional epithelium, which originates from oral epithelium, attaches to the implant by basal lamina. The biological width (CT and epithelium) around implant is slightly greater than teeth, 2.62mm and 2.04mm, respectively. The peri-implant sulcus consists of non-keratinized epithelium and junctional epithelium. An inner zone of the connective tissue that is in contact with the implant is composed of dense circular fibers without blood vessels, while the outer zone is less dense with collagen fibers and blood vessels. Peri-implant fibers run in a parallel direction, unlike fibers around teeth. Blood supply to the peri-implant soft tissue comes from terminal branches of bone periosteum, forming
crevicular plexus adjacent to the junctional epithelium. Thus, with these characteristics, peri-implant soft tissue is a more scar-like fibrotic tissue compared to periodontal tissue.

Healing at the bone level is defined as osseointegration. Similar to soft tissue healing, it is divided into three distinct phases: (1) osteoconduction; (2) De novo bone formation; and (3) bone remodeling. Bone healing starts with formation of a coagulum, followed by infiltration of inflammatory cells that initiate removal of necrotic tissue. This granulation tissue is eventually replaced by a provisional matrix. Osteoconduction relies on migration of differentiating osteogenic cells to the implant surface through a connective tissue scaffold. De novo bone formation results in mineralized interfacial matrix that is laid down on the implant surface, which is determined by the implant surface topography. Bone formation starts during the first week of healing. Bone immediately adjacent to the implant surface is resorbed and replaced with new viable bone. Newly formed woven bone replaces lamellar bone, through bone remodeling. Through these phases of osseointegration, direct structural and functional connection between living bone and surface of the implant occurs.

**Diagnostic parameters**

Different phases of osseointegration are well understood; however, evaluation of peri-implant wound healing is limited to various clinical parameters. Diagnostic tools to evaluate the health of dental implants include assessment of plaque accumulation, mucosal conditions including gingival bleeding, suppuration, edema, and peri-implant probing depth. Implant mobility, discomfort, and recent addition of resonance frequency analysis used to evaluate implant stability are currently available clinical diagnostic tools.
It is recommended that radiographic evaluation should be included to clinical parameters to evaluate and compare alveolar crest bone level prior to and following mechanical loading.\textsuperscript{10} The sensitivity and specificity of these diagnostic tools in detecting peri-implant pathology have been discussed.\textsuperscript{11-17}

Implants with peri-implantitis are strongly associated with no accessibility/capability for proper hygiene.\textsuperscript{18, 19} Experimental plaque accumulation studies show stronger inflammatory response elicited around implants than teeth,\textsuperscript{10} resulting in loss of supporting tissues and large inflammatory cell infiltrate that extends to the bone crest.\textsuperscript{20}

Peri-implant probing depths are slightly greater than probing depth around natural dentition even in health (2.55mm, 2.02mm, respectively).\textsuperscript{21-24} This was speculated to be due to weaker (shorter and thinner) junctional epithelium. It has been reported that even in mild marginal inflammation, the probe tip penetrates deeper around implants than teeth.\textsuperscript{22} Probing depth around implants may relate to pre-existing flap thickness before implant insertion and/or to presence of local disease.\textsuperscript{25} It may also depend on depth of implant placement in relation to bone crest.\textsuperscript{125, 126} Once formed, peri-implant probing depths are stable from 4 to 12 weeks after one-stage implant placement.\textsuperscript{26} It has been reported that mucosal seal is stable by day 5 of healing and probing can be performed to evaluate soft tissue characteristics as early as first week of healing.\textsuperscript{27} Increased probing depth during healing or maintenance period may reflect bone loss around implants and is associated with greater incidence of anaerobic bacteria\textsuperscript{28} and higher risk of developing peri-implantitis.\textsuperscript{29} Lang et al. (1994)\textsuperscript{30} suggested that probing around non-submerged implants is a good technique to assess the health of the peri-implant tissues.
The necessity of peri-implant keratinized mucosa to maintain health has been controversial. Lack of keratinized gingiva (KG<2mm) is negatively associated with gingival index, plaque index, bleeding on probing, probing depth, and mucosal recession.\textsuperscript{21, 31-37} However, it does not warrant poor hygiene and inflammation.\textsuperscript{21} Some studies show significant association with higher radiographic bone loss\textsuperscript{33}, while others show no association\textsuperscript{35}. Systematic reviews show that quantitative advantages of having KG is minimal\textsuperscript{38} and does not influence the survival rate of dental implants\textsuperscript{4, 39}.

Suppuration or an abscess around implant indicates peri-implant disease,\textsuperscript{28} involving either soft tissue alone or both soft and hard tissues. This may compromise survival of the implant.

A healthy implant may move less than 75 µm.\textsuperscript{28} A clinically mobile implant, however, indicates presence of connective tissue between implant and bone, thus lack of osseointegration. Implant stability can be evaluated by removal torque value, Periotest\textsuperscript{®}, or Resonance Frequency Analysis (RFA). Removal torque value can be only used in \textit{in vitro} and \textit{in vivo} experimental models. Periotest\textsuperscript{®} and RFA, on the other hand, permit non-invasive methods to assess implant stability. For RFA, a small bending force that mimics occlusal forces at a minimal magnitude from a hand-held device is directed towards a magnetic transducer that is directly attached to the implant. This causes vibratory movement in two directions, and the frequency of oscillation depends on the stiffness of bone-implant attachment. This information is converted from Hertz to implant stability quotient (ISQ) that ranges from 1 to 100, with high values indicating high stability. ISQ of stable implants range 57-70 at time of implant placement.\textsuperscript{40} Another study reported that ISQ greater than 47 indicated implant stability.\textsuperscript{104} However, RFA is
not a reliable diagnostic tool to identify mobile implants. A significantly lowered RFA compared to the values obtained at implant placement is detected in failing implants, which decreases continuously until failure/mobility. During osseointegration, lower ISQ at 3 weeks post-op is reported compared to ISQ at implant placement, mainly due to callus formation. ISQ, then, increases continuously to baseline measurement or greater until 12 weeks.

ISQ reading may be affected by bone density and position in the jaw and arch. Some authors report that implant length is not a significant influence on the ISQ value, while others disagree. Furthermore, no significant difference in ISQ values of 4.8mm and 4.1mm diameter implants were reported. However, ISQ values of 3.3mm diameter implants are lower. Mean ISQ value for SLActive implants, primarily in posterior areas, in both mandible and maxilla, were reported to be 75.7 (range 65.3-81.3) at placement; 71.4 at 3 weeks; 73.5 at 4 weeks; 78.8 at 12 weeks post-op. In addition, gender of the patient is reported to affect RFA with higher ISQ values in men than women. There is a significant correlation of RFA to insertion torque values. Positioning of RFA device does not seem to affect the ISQ values. It has high repeatability and reproducibility. Therefore, RFA provides easy to use, non-invasive, chair-side evaluation of implant stability during healing.

Careful radiographic assessment can help monitor hard tissue changes around the implant. Yearly radiographic marginal bone loss after the first year of loading ranges from 0 to 0.2mm. According to Laurell and Lundgren (2011), implant system used in current study* had marginal bone level change of average 0.48mm over 5 years.

* Straumann USA, LLC. Andover, MA, USA
Therefore, some marginal bone change is expected. However, progressive bone loss, concurrent to pain and mobility of the implant, are signs of implant failure, which necessitates implant removal.

**Peri-implant sulcus fluid analysis**

Peri-implant crevicular fluid (PICF) is osmotically mediated transudate from the crevicular plexus in the connective tissue adjacent to the junctional epithelium in health, which contains cytokines corresponding to innate immune response. In diseased tissues, this becomes into inflammatory exudate, due to vascular hyperpermeability that leads to increase in osmotic pressure of the fluid and inflammatory cytokines towards the sulcus.\(^\text{23}\) PICF of healthy functioning implants has significantly higher concentrations of interleukin-8 (IL-8), tumor necrosis factor (TNF)-α, and a tendency for higher concentrations of IL-6, IL-10, and IL-12, compared to gingival crevicular fluid (GCF) of healthy teeth.\(^\text{53}\) However, crevicular fluid volume of an un-restored implant is lower than of teeth after healing.\(^\text{1}\) The flow and concentration is positively correlated to conditions of the peri-implant tissues.\(^\text{23, 54-56}\) such as plaque index, gingival index, bleeding on probing, and pocket depth.\(^\text{23, 24}\) Therefore, analysis of PICF, in addition to clinical parameters, can be a helpful tool in discerning the implant and peri-implant tissue health during healing and maintenance.

Content of crevicular fluid is a mixture of inflammatory mediators and products, host derived enzymes and inhibitors, host-response modifiers, tissue break down products, and putative pathogens.\(^\text{57, 58}\) There are some controversial data available through literature on PICF cytokine contents in health and disease. The discrepancies
may be related to several factors including sampling technique, sampling time, description of health and disease, method of presentation of data in concentration, total amount, or ratio. Among the pro-inflammatory cytokines, the role of interleukin-1β (IL-1β) and TNF-α in periodontal tissue destruction are well known.\textsuperscript{59} Both of these cytokines are released by activated junctional epithelium upon bacterial insult during periodontal disease progression,\textsuperscript{60} and show positive correlation to each other in PICF of healthy implants.\textsuperscript{56} IL-1β activates osteoclasts, stimulates bone resorption, and induces tissue-degradating proteinases. TNF-α has similar but less potent properties than IL-1β. It is the main mediator in response to Gram-negative bacteria. The concentration of TNF-α correlates to amount of bacteria and stage of inflammation.\textsuperscript{56} IL-1β and TNF-α concentrations are positively correlated with bone loss around implants and teeth,\textsuperscript{61} which synergistically enhance bone resorption\textsuperscript{62}. Concentration of IL-1β does not differ between healthy implants and healthy teeth.\textsuperscript{54, 63} High level of IL-1β are also detected in implants with advanced inflammation (GI>1) of the supporting tissues compared to healthier implants although there are some controversial reports.\textsuperscript{54, 57, 64} It has been documented that levels of IL-1β\textsuperscript{65} and TNF-α increase with severity of disease around the implant,\textsuperscript{56, 57} while others reported that there was no significant difference in IL-1β concentrations among healthy implants and those with peri-implantitis.\textsuperscript{17, 63, 66} A study reported that the TNF-α levels are lower compared to IL-1β in PICF of inflamed tissues, and are not correlated with clinical parameters.\textsuperscript{64} Anti-infective surgical therapy for peri-implantitis has shown to significantly decrease the level of TNF-α after 3 months.\textsuperscript{68, 69} During early phases of healing of one-stage implants, IL-1β concentration, correlated with gingival index, increases at 1-week postoperatively.\textsuperscript{70}
Interleukin-6 is another potent pro-inflammatory cytokine that is released by macrophages, endothelial cells, T-cells, plasma cells, and fibroblasts that contribute to periodontal tissue destruction. It is responsible for collagen resorption of gingival tissues and activation of osteoclasts. The level of IL-6 is also increased around implants with peri-implantitis. However, this is controversial since there are studies reporting no differences in PICF IL-6 concentrations between healthy and diseased implants. IL-6 is also a well-known cytokine released as a wound healing response to acute trauma. IL-6, in conjunction to IL-10, can act as anti-inflammatory cytokine by inhibiting production of IL-1β and TNFα. In a study comparing healthy implants to those with implantitis, no significant differences in levels of IL-6 and IL-10 were found between groups. Interleukin-10 is an anti-inflammatory cytokine that downregulate matrix metalloproteinase (MMPs) and upregulate tissue inhibitor of metalloproteinases (TIMPs), which inhibit collagenase activity. A recent study reported tendency for higher level of IL-10 in healthy implants than those with implantitis.

Endothelial cells release interleukin-8 (IL-8), which acts as a chemokine to selectively recruit and activate neutrophils for innate immune response. In healthy functioning implants, PICF IL-8 concentrations are higher than around teeth. There is a positive correlation between IL-6 and IL-8 in implantitis group, showing high expression in PICF of diseased compared to in health. Also, in relation to healing, PICF IL-8 concentration is increased at 1-week postoperative healing following one-stage implant placement. Macrophage inflammatory protein-1 (MIP-1) is a chemokine that recruits osteoclast progenitors and contributes in various pathophysiological conditions. Chemokines are classified by presence (CXC) or absence (CC) of an amino acid at a
specific location in the molecular structure.\textsuperscript{77} MIP-1\(\alpha\) and MIP-1\(\beta\) are ligands for CCR5, a chemokine receptor expressed on monocytes/macrophages for Th1 cell type response. Both are present in inflamed gingival samples from patients with chronic periodontitis.\textsuperscript{77} However, another study reported no significant difference in the GCF levels of MIP-1\(\beta\) between sites with periodontal disease and healthy sites.\textsuperscript{78} Levels of MIP-1\(\alpha\) and IL-8, positively correlated to each other, have been reported to increase in PICF with increase in degree of inflammation around implants.\textsuperscript{56}

Monocyte chemoattractant protein (MCP)-1 is one of the most potent chemoattractant for monocytes/macrophages.\textsuperscript{77,79} Level of MCP-1 is higher in crevicular fluid of subjects with chronic periodontitis than healthy subjects.\textsuperscript{80} Like MCP-1 and MIP-1, Interferon-inducible protein 10 (IP-10) attracts monocytes/macrophages and T-helper cells. It is a ligand for CXCR3, expressed on Th1 cells, and found in inflamed gingival samples from patients with chronic periodontitis.\textsuperscript{77} In GCF samples, IP-10 is expressed more frequently and at higher levels in periodontal diseased sites than healthy sites.\textsuperscript{78}

Growth factors are small proteins that serve as signaling molecules that are mitogenic and angiogenic.\textsuperscript{81} They are essential in regulation of bone tissue during healing. Among the main growth factors that are involved in wound healing are vascular endothelial growth factor (VEGF), platelet-derived growth factors (PDGF), and fibroblast growth factor (FGF). One study reported that compared to patients with stable implants, patients with failing implants exhibited higher levels of PDGF within PICF of both failing and stable implants.\textsuperscript{82} VEGF plays role in angiogenesis and regulation of bone remodeling, attracts endothelial cells and osteoclasts, and stimulates osteoblast differentiation.\textsuperscript{83} In periodontal patients, there are higher VEGF levels in diseased sites
than healthy sites.\textsuperscript{84} FGF also plays role in bone remodeling. FGF-4 is present more frequently and at higher levels in sites with periodontal disease than healthy sites.\textsuperscript{78} Despite their important role in tissue remodeling, diagnostic values of growth factors relating to disease or healing around implants is not well understood.

Most of the information related to wound healing cytokines is based on studies on dermal skin wounds.\textsuperscript{85-87} The introduction of a dental implant device, presence and changes in saliva flow and microflora may differentially impact peri-implant wound healing compared to dermal wound healing. Our previous work showed that it is possible to detect the acute trauma response to the insertion of dental implant device by investigating the content of peri-implant crevicular fluid at early phases of healing.\textsuperscript{1} The response that we originally reported was specific to surgical site. Thus, the purpose of the current study was to determine whether a site-specific differential response to surgical trauma can be detected when a single surgeon simultaneously creates two similar wounds by using same surgical procedure and implant system at two quadrants within an oral cavity.
Working Hypothesis:

It is possible to detect site-specific characteristics of acute surgical trauma when two peri-implant related wounds are simultaneously created within the same oral cavity.

Specific aims:

1- To determine site-specific clinical characteristics of peri-implant wounds.

2- To determine site-specific changes in peri-implant crevicular fluid content during early wound healing at protein level.

Significance of the study:

The ultimate goal of this study is to determine site-specific differences in clinical parameters and crevicular fluid analysis of one-stage implants during early phase of healing. The results may help in developing modalities in evaluation of healing after dental implant insertion.
CHAPTER II

MATERIAL AND METHODS

Study population

Patients with two missing teeth, each located at two separate posterior sextants, who were interested in implant supported dental restorations, were recruited for this study. Inclusion criteria were periodontally and systemically healthy adults with no known risk factors for delayed type of wound healing, single edentulous tooth site at two different posterior sextants that were scheduled for implant placement in the same visit, following one-stage implant placement protocol. Exclusion criteria included smoking habit, systemic diseases possibly affecting the healing process, need for antibiotic prophylaxis prior to surgery, absence of keratinized tissue at the implant site, need for simultaneous hard or soft tissue grafting, and initial ISQ values ≤ 45 at time of implant placement. Subjects requiring separate surgical appointments for each site were also excluded. Exit criteria were existence of any healing complications, such as infection, soft tissue covering healing abutment, that require additional treatment during observation time, and non-compliance to follow-up visit schedule. Study protocol was reviewed and approved by the Internal Review Board at the Ohio State University. Subjects signed a written consent form prior to their enrollment.
Study Design

The study design was a prospective observational trial with a four-week follow-up period. Figure 1 is a flow chart presenting different phases of study design. Briefly, at baseline, demographical data, including age, gender, and race/ethnicity, were collected. Medical and dental histories were reviewed to ensure patient’s eligibility for the study. Full-mouth plaque and gingival indices were recorded by using Ramfjord teeth. In addition, PI and GI were obtained for the surgical sites. Gingival Crevicular Fluid (GCF) was collected from adjacent teeth and Probing Depth (PD) was recorded at six surfaces of adjacent teeth. During surgery, surgical measurements including the width of keratinized tissue at mid-buccal surface of the edentulous site, flap thickness and buccal bone thickness at mid-buccal of osteotomy site were measured, and initial stability of the implant was determined by using Resonance Frequency Analysis (RFA). In addition, soft tissue biopsy was obtained from the scalloped incision used to adapt soft tissue around healing abutment as part of one-stage surgical procedure. Follow-up appointments were at 1 week and 4 weeks post-operatively. Clinical measurements (PI, GI, PD, CF collection from implant and adjacent teeth) were repeated. In addition, a 3 mm-punch biopsy was obtained from distal interdental papillary space between distal tooth and implant. The biopsy was obtained from one site at 1 week and from the other site at 4 weeks. Finally, RFA measurements were repeated at 4 weeks post-operative visit.
Surgical protocol

All surgeries were performed by second and third year residents in Graduate Periodontics clinic at the Ohio State University from 2010 to 2012. Routine surgical protocol included local anesthesia via administration of 2% lidocaine hydrochloride with 1:100,000 epinephrine. Intrasulcular and crestal incisions were followed by elevation of mucoperiosteal flaps buccally and lingually. Wound size was limited to edentulous site and two adjacent teeth. After degranulation and irrigation, osteotomy was performed by using specific surgical kit† and by following manufacturer recommendations. Dental implant devices from a single company‡ were inserted to the osteotomy site by using a handpiece, ratchet, or a combination of both. Implant diameter and length varied based on anatomical location. During surgery, amount of keratinized tissue from mucogingival junction to incision line at mid-buccal of edentulous site was measured, using a periodontal probe§, while soft and hard tissue thickness was measured at mid-buccal of osteotomy site approximately 1 mm apical to alveolar crest, using a caliper**. Implant stability was measured and recorded by using Resonance Frequency Analysis††. A connective tissue sample (approximately 3x3mm in width and length) from the periosteum side of the flap was obtained with a scalpel and stored in a sterile vial, which was immediately frozen in liquid nitrogen and kept at -80°C. Healing abutments were

†† Straumann USA, LLC. Andover, MA, USA
‡ SLActive bone level implants, Straumann USA, LLC. Andover, MA, USA.
§ UNC-15 probe, Hu-Friedy, LLC. Chicago, IL, USA
** Boley Gauge BG, Hu-Friedy, LLC. Chicago, IL, USA
†† Osstell ISQ™, Type 41 and 42 SmartPeg. Osstell USA. Linthicum, MD, USA.
placed in implants and flaps were sutured with polyglycolic acid sutures according to one-stage implant protocol. Patients were instructed to refrain from mechanical brushing in the surgical areas for 10 days and use 0.12% chlorhexidine gluconate daily. Pain medications included ibuprofen 600mg, acetaminophen 500mg and/or acetaminophen 500mg/hydrocodone 5mg. Patients were given post-surgical antibiotic regime based on clinician’s discretion.

Clinical Data Collection

Clinical Parameters

Probing depths were measured as millimeters at six surfaces for newly placed implants and adjacent teeth at baseline, 1 and 4 week post-operatively, using standardized probes.

Width of keratinized tissue at mid-buccal surface of the edentulous sites was measured at time of implant placement from mucogingival junction to the crestal incision. Standardized periodontal probe was used to report in millimeters.

The full mouth plaque index was measured by taking the average of scores from four surfaces of teeth #3, 9, 12, 19, 25, 28. Adjacent tooth was used if one of previously mentioned teeth were missing. This measurement was collected at baseline, 1 and 4 weeks post-operatively. The plaque index of the surgical site was collected at baseline prior to surgery. Similar to full-mouth plaque index, average of plaque scores from four surfaces of adjacent teeth were recorded. The measurements were repeated at 1 and 4

‡‡ Polyglycolic acid (PGA) Perma Sharp Sutures. Hu-Friedy, LLC. Chicago, IL, USA.

§§ UNC-15 probe, Hu-Friedy, LLC. Chicago, IL, USA.
weeks post-operatively from implant sites and adjacent teeth sites in the surgical field. A modified plaque index (mPI)\textsuperscript{90} assessed smooth marginal surface of the implant, while the original plaque index used for natural dentition examined plaque adhering to the free gingival margin, within the gingival pocket, and adjacent area of the tooth. Average of these mPI and PI for surgical sites were recorded.

Similar to plaque index\textsuperscript{89}, full mouth gingival index\textsuperscript{88} assessed four surface of teeth #3, 9, 12, 19, 25, 28 for an average score. This measurement was collected at baseline, 1 and 4 weeks post-operatively. Same method was used to assess teeth in the surgical field and the Modified Sulcus Bleeding Index (mBI)\textsuperscript{90} was used to assess tissues around the implants. While the original gingival index used for natural dentition entails color, texture, bleeding, ulceration, and hypertrophy in the gingival tissues, mBI focuses on the extent of bleeding when a periodontal probe is passed along the gingival margin adjacent to the implant. Average of these mBI and GI for surgical sites were recorded.

**Gingival and Peri-implant crevicular fluid collection**

Crevicular fluid collection was performed around newly placed implants at one and four weeks post-operative appointments; while GCF collection around surgically manipulated adjacent teeth was completed prior to surgery and at one and four weeks post-operatively. Briefly, following isolation of the surgical site with cotton rolls and gentle air-drying to prevent saliva contamination, a sterile paper strip *** was gently introduced at the orifice of sulcus for 20 seconds. Collected volume was determined by

\[ *** \text{Periopaper, Oralflow Inc. Smithtown, NY, USA} \]
using a calibrated electronic volume quantification unit. Four strips were used for each peri-implant wound sampling and periodontal wound sampling. Strips were placed in assigned sterile cyrotubes and kept on ice until stored at -80°C. Any strip with blood contamination was discarded. Crevicular fluid was collected prior to probing, local anesthesia, and gingival biopsy to eliminate contamination.

**Gingival Biopsy Sampling**

A connective soft tissue biopsy (approximately 3x3mm in width and length) was obtained from the periosteum site of the flap with a scalpel at time of the surgery for each implant site. A gingival biopsy of 3mm diameter was obtained using punch biopsy after local anesthesia from one site at 1 week and from the second surgical site at 4 weeks post-operatively. Collected soft tissue biopsies were stored in sterile vials and immediately frozen in liquid nitrogen and stored at -80°C for future gene expression analysis.

**Resonance frequency analysis (RFA)**

Implant stability was determined by using RFA at the time of placement and at 4 weeks post-operatively. The area was dried and healing abutment was unscrewed. An appropriate type transducer for the implant design and size was screwed into the implant and Implant Stability Quotient (ISQ) was obtained at least from two directions.

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††† Periotron 8000®, Oralflow Inc. Smithtown, NY, USA

+++ 3mm Biopsy Punch. Miltex Inc. York, PA, USA.

§§§ Osstell ISQ™, Type 41 and 42 SmartPeg. Osstell USA. Linthicum, MD, USA.
(buccal and lingual). The average of these two numbers was used for data analysis. Any
two values that differed more than 5 units were repeated to ensure the values. Implants
with initial ISQ values equal or smaller than 45 had to be placed following the two-stage
surgical protocol and were excluded from the study.

Laboratory Analysis

Crevicular Fluid Sample Preparation

Sterilized PCR tubes with holes at the bottom were prepared, which were placed
inside of sterile Eppendorf tubes. Frozen paper strips were thawed on ice for 15 minutes.
The orange/waxed part of the strip was cut with sterile instruments and discarded. The
filter portion, which contained crevicular fluid, was placed into PCR tubes with holes at
the bottom. Strips were soaked in 200 µl ice cold sterile PBS, incubated in ice for 15
minutes with occasional vortexing. All samples were centrifuged at 14,000 rpm for 10
minutes. Following a second centrifugation, approximately 160µl supernatant collected
inside of Eppendorf tube was procured while paper strips were discarded. This
supernatant was used to load multiplex plates.

Multiplex bead-based assay

The multiplex bead-based assay**** was used to detect the presence and the
amount of 19 cytokines, chemokines, and growth factors within crevicular fluid samples
including PDGF, IL-1β, IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-17, Eotaxin, FGFb, G-CSF,
IFN-γ, IP-10, MCP-1, MIP-1β, TNF-α and VEGF. Specific kit designated as human

**** Bio-Plex Pro™ Assays, Bio-Rad Laboratories, Inc.
cytokine group (Group I) was used for these experiments. The cytokine reagent kit includes assay buffer, antibody diluents, streptavidin-PE and a 96- well filter plate. Briefly, the assay uses Luminex multi-analyte profiling (xMap) technology. This technique uses digital signal processing capable of classifying polystrine 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 fluorescence of the cytokine of interest is measured and compared to a standard curve obtained from cytokine of known concentrations. The digital processor presents the data as the median fluorescence intensity and concentration (pg/ml).

**Statistical analysis**

Data were analyzed by using both GraphPad Prism version 5†††† and SAS version 9.2 softwares‡‡‡‡. Repeated Measures ANOVA was used to fit the model. Sandwich estimator was used to control the correlation that might exist in the data associated with the same subject. T-test was performed within the ANOVA to make pair-wise comparisons. Multiple comparison adjustment was performed, using Bonferroni adjustment method, to control the overall error rate. Correlations among clinical parameters and cytokines were analyzed using Spearman Correlation Coefficient. Results were considered significant at P value ≤0.05.

†††† GraphPad Prism, GraphPad Software Inc. La Jolla, CA, USA

‡‡‡‡ SAS version 9.2, SAS Institute Inc. Cary, NC, USA
CHAPTER 3

RESULTS

Study population

A total of 27 subjects fulfilled the inclusion criteria and were recruited for the study. Twenty-two subjects completed the study. Five subjects were excluded due to conversion of surgical protocol to two-stage protocol (N=2), technical complication during surgery (N=1), implant failure at week 1 post-operatively (N=1), and development of herpetic lesions around the surgical area at week 1 (N=1). Of the twenty-two subjects that completed the study, four subjects had one of the implants that failed at week 4. For subjects with implant failures, surgical interventions were executed.

The patient demographical data and location of implants are given in Table 1. Study population included 22 subjects, entailing 13 female and 9 male subjects with mean age of 55±3 years. Each subject received implants at two different quadrants to replace missing posterior teeth, creating two similar surgical sites in an oral cavity. Therefore, results of this study encompass a total of 44 implants in 22 patients. Each surgical area was retrospectively assigned as either site A or site B. For sites A, twelve implants were in maxillary posterior and ten implants were in mandibular posterior area. For sites B, eight implants were located in maxillary posterior and 14 implants were in mandibular posterior area. Each of sites A and B had ten implants that replaced molars, while 12 replaced premolars. Eleven of site A and nine of site B were grafted prior to implant insertion appointment.
Clinical Measurements

Mean flap thickness was 2±0.1 mm and 1.9±0.12 mm for site A and site B, respectively (Figure 2). Mean buccal bone thickness was 2.5±0.2 mm and 2.4±0.15 mm for site A and site B, respectively. There was no statistically significant difference between wound sites for these two surgical parameters (P>0.05). The mean amount of buccal keratinized tissue was 5±0.6 mm at site A and 4.3±0.5 mm at site B with no statistically significant difference between sites (P>0.05).

Mean pre-surgical probing depth of adjacent teeth was 2.1±0.13 mm for site A and 2±0.13 mm for site B, with no statistically significant differences between sites (Figure 3; P>0.05). In sites A, probing depths of adjacent teeth were 2.7±0.13 mm and 2.4±0.12 mm at 1 week and 4 weeks post-operatively (P=0.01), while peri-implant probing depths were 3.1±0.17 mm and 3±0.2 mm at 1 and 4 weeks (P>0.05). For sites B, probing depths of adjacent teeth were 2.5±0.1 mm and 2.3±0.1 mm at 1 week and 4 weeks post-operatively (P=0.002), while peri-implant probing depths were 3.2±0.16 mm and 3±0.2 mm at 1 and 4 weeks (P>0.05). The difference between probing depths of implants to teeth was statistically significant at 4 week for site A (P=0.03) and site B (P=0.002).

Plaque accumulation and soft tissue characteristics during healing were recorded by using PI and GI. Baseline PI prior to surgery was higher at both surgical sites compared to plaque accumulation recorded around Ramfjord teeth (e.g. representation of full mouth) (0.53±0.1 [0.5 (0-2)] and 0.55±0.1 [0.55 (0-2)] for site A and site B, respectively; and 0.45±0.1 [0.3 (0-2)] for full mouth) (Figure 4). This difference was not statistically significant (P>0.05). Plaque accumulation was well controlled throughout the study period (PI<0.7) for the entire mouth, as well as at each surgical site. A decrease
in plaque accumulation throughout the study was noted at both surgical sites (from 0.53±0.1 to 0.34±0.06 (median 0.5 [0-2] to 0.3 [0-2]) for site A and from 0.55±0.1 to 0.33±0.1 (median 0.55 [0-2] to 0.3 [0-2]) for site B) with no statistically significant differences between sites (P>0.05).

Soft tissue characteristics including redness, edema, and bleeding were defined by Gingival Index (GI). Mean full-mouth GI was 0.31±0.07 (median 0.17 [0-1.2]) prior to surgery and did not differ significantly throughout the study period (Figure 5). Pre-surgical GI at surgical sites were slightly higher compared to baseline GI for whole mouth (0.4±0.07 (0.38 [0-1.5]) and 0.5±0.1 (0.44 [0-2]) for site A and B and, 0.31±0.07 (0.17 [0-1.2]) for whole mouth, respectively; P>0.05). As expected, GI increased at surgical sites at 1 week post-operatively (0.5±0.08 (0.5 [0-1.5]) for site A and 0.6±0.09 (0.58 [0-1.5]) for site B). The difference between two sites was not statistically significant (P>0.05). These values significantly decreased at both sites by 4 weeks post-operatively (0.3±0.05 (0.25 [0-0.81]) for site A and 0.3±0.07 (0.25 [0-1.1]) for site B). However, this decrease was also not statistically significant for either sites (P>0.05) (Figure 5).

**Resonance Frequency Analysis**

Implant stability was assessed by Resonance Frequency Analysis (RFA) (Figure 6). Mean Implant Stability Quotient (ISQ) immediately after implant placement for site A was 69±1.3, while it was 73±1 for site B (P=0.03). By 4 weeks post-operatively, ISQ values increased to 71±1.4 for site A and decreased to 69±2.4 for site B. The difference
between two sites was not statistically significant at week 4 mainly due to higher standard error value for site B (P>0.05).

**Crevicular Fluid Volume**

Gingival and peri-implant crevicular fluid (GCF and PICF) volume, calculated as µl by using a calibration curve obtained through measurements of known volumes of distilled water is presented in figure 7. Baseline GCF volumes obtained from teeth in surgical sites A and B were very similar (0.8 ±0.1 µl and 0.8±0.07 µl for sites A and B, respectively). As anticipated, CF volumes for both implant and surgically manipulated adjacent tooth sites increased significantly by week 1 (1.7±0.2 µl for PICF at site A and 1.1±0.13 µl for PICF at B; 1.4±0.2 µl for GCF at site A and 1.1±0.15 µl for GCF at site B; P>0.05). The difference between sites A and B for PICF volume was statistically significant at week 1 (P=0.05). By week 4, GCF volumes decreased to baseline levels around surgically manipulated tooth (0.9±0.1µl at site A and 0.74±0.1 µl at site B; P>0.05). Significant decrease in PICF volume was also noted at peri-implant sites (0.5±0.1 µl and 0.54±0.08 µl for site A and site B, respectively). The difference in PICF volume between week 1 and week 4 was statistically significant only at site A (P=0.002) (Figure 7).

**Expression of Cytokines**

Multiple bead-based assay system was used to isolate up to 19 pro-inflammatory cytokines within crevicular fluid during early phases of healing. The following cytokines were detected although some were expressed at significantly lower level at all time
points: PDGF, 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-1β, TNFα, and VEGF (Table 2).

Interleukin-1β is a pro-inflammatory cytokine, responsible for inducing bone resorption. Baseline GCF levels of IL-1β did not differ significantly between two wound sites (Figure 8; 18±4 pg and 17±5 pg for site A and site B, respectively; P>0.05). At 1 week post-operative observation period, PICF levels of IL-1β was higher than GCF levels at site A (Figure 8; 33±8 pg compared to 14±2 pg for site A (P=0.65), 20±6 pg compared to 14±3 pg for site B (P>0.05)) with no statistically significant differences between sites. By week 4, PICF IL-1β expression decreased at both sites A and B (7±2 pg at site A and 11±3 pg at site B). This decrease was not statistically significant compared to 1 week PICF IL-1β levels at site A (P=0.08) and at site B (P=0.17) and it was similar to baseline GCF IL-1β levels (P>0.05) at both sites (Figure 8).

Tumor necrosis factor-α (TNF-α) has similar functions to IL-1β by inducing bone resorption. Baseline TNF-α levels in GCF of this patient group was close to zero. At one week, a 3 to 5 fold increase was noted only at implant sites and not at surgically manipulated adjacent tooth sites (Figure 9; 1.1±0.4 pg and 5±1 pg for GCF and PICF levels at site A, respectively; and, 1.2±0.4 pg and 3.4±1.3 pg for GCF and PICF levels at site B, respectively). These differences between GCF and PICF levels were not statistically significant (P>0.05 for both sites). This acute response to surgery disappeared by week 4. The differences between week 1 and week 4 were not statistically significant following adjustment of P values for multiple comparisons (P>0.05 for both sites).
Interleukin-6 is another pro-inflammatory cytokine responsible for osteoclast activation. In addition, it induces collagen resorption in the gingiva. IL-6 was barely detected at baseline around teeth at both sites (0.2±0.1 pg and 0±0 pg at site A and site B, respectively) (Figure 10). Surgical manipulation induced IL-6 expression at both tooth and implant sites (15±4 pg and 13±5 pg for GCF samples obtained from sites A and B, respectively; 34±8 pg and 29±7 pg for PICF samples obtained from sites A and B, respectively). The difference between IL-6 levels in GCF and PICF was more than 2 fold for both sites (P>0.05 for site A and P=0.002 for site B). At fourth week post-operative observation time, IL-6 expression decreased significantly to baseline levels for GCF levels (0.4±0.2 pg and 2±1 pg for site A and B, respectively; P=0.02 for site A and P>0.05 for site B). There was also a decrease in PICF IL-6 levels at both sites by 4 weeks (5±4 pg and 7±4 pg for site A and B, respectively; P=0.06 for site A and P=0.02 for site B). The differences between two implant sites were negligible.

Interleukin-1 receptor antagonist (IL-1ra) competitively binds to IL-1 receptors and inhibits IL-1 activity. The data presented at both table 2 and figure 11 show that IL-1ra is highly expressed in GCF even prior to surgery (5890±510 pg and 4702±349 pg at site A and site B, respectively). The difference observed at baseline GCF IL-1ra level between site A and site B was not statistically significant (P>0.05). Surgical manipulation of tooth sites did not have significant effect on GCF levels of IL-1ra by week 1 (5106±818 pg and 4565±747 pg at site A and site B, respectively; P>0.05). IL-1ra expression in PICF following first week of healing was 3 fold less than GCF level at site A and almost 2 fold less than GCF level site B (1709±211 pg and 2511±461 pg as PICF IL-1ra levels at site A and site B, respectively). The difference between GCF and PICF
levels at week 1 were statistically significant for site A (P=0.002) while it was not statistically significant for site B (P>0.05). A slight increase in IL-1ra level was noted at 4 week healing for PICF levels of IL-1ra at site A, while no difference was detected at site B (2899±577 pg and 2266±447 pg for site A and B, respectively; Figure 11). The difference between GCF and PICF levels at week 4 was statistically significant for both site A and site B (P=0.04 and P=0.002, respectively).

Interleukin-8 is a chemo-attractant for neutrophils. There was no significant difference between sites A and B in IL-8 expression within GCF prior to surgery (30±3 pg at site A and 29±9 pg at B; P>0.05; figure 12). At one-week post-operative healing time, a noticeable increase in IL-8 levels was seen at both newly created implant sites and surgically manipulated adjacent teeth sites (71±17 pg and 81±19 pg for GCF levels at site A and site B, respectively; 317±78 pg and 203±64 pg for PICF levels at site A and site B, respectively). The difference between PICF and GCF levels of IL-8 was 4.5 fold for site A and 2.5 fold for site B. However, this difference was not statistically significant for either sites following adjustments of P values for multiple comparisons (P>0.05). IL-8 expression at four week post-operatively were similar to baseline values with no statistically significant differences between groups and sites (Figure 12; 38±7 pg and 50±2 pg for GCF levels at site A and site B, respectively; 17±5 pg and 44±19 pg for PICF levels at site A and site B, respectively) (P>0.05).

Granulocyte-Colony Stimulating Factor (G-CSF) is a pro-inflammatory cytokine that modulates fibroblast migration. Baseline GCF levels for G-CSF was in the range of 7 to 8 pg with negligible fluctuations in 1 week and 4 weeks (Figure 13; 8±1pg prior to surgery, 9±1 pg at week 1, and 7±1 pg at week 4 for surgically manipulated tooth at site
A; 7±1 pg prior to surgery, 7±1 pg at week 1, and 7±1 pg at week 4 for surgically manipulated tooth at site B). There was no statistically significant difference for GCF levels at any time for both site A and site B (P>0.05). There was a 1.8 fold increase in G-CSF total amount within PICF at 1 week at site A, while it increased by 1.6 fold within PICF at site B (Figure 13; 14±3 pg for site A and 11±3 pg for site B). These levels were not statistically different from adjacent GCF levels at week 1 for both sites (P>0.05). PICF G-CSF levels decreased to GCF G-CSF levels by week 4 (4±1 pg and 6±1 pg for site A and site B, respectively). The differences observed between week 1 and week 4 were not statistically significant for both sites following adjustment of P values for multiple comparisons (P>0.05).

Interferon gamma induced protein-10 (IP-10) is a chemokine that attracts monocytes/macrophages and T-helper cells. Baseline GCF levels for IP-10 ranged from 19 to 28 pg (Figure 14; 28±8 pg at site A and 19±5 pg at site B). A decrease in GCF IP-10 levels was noted following surgical manipulation of teeth at both sites after a week of healing (13±3 pg and 14±6 pg at site A and site B, respectively). PICF IP-10 levels were 7±2 pg at site A and 10±5 pg at site B, at one week post-operative healing period. At week 4, a slight increase within both GCF and PICF IP-10 levels at site A was noted, while these values decreased slightly for site B (18±5 pg for GCF and 11±6 pg for PICF at site A; 12±3 pg for GCF and 5±2 pg for PICF at site B). The differences between groups and within group for different times were not statistically significant (P>0.05).

Macrophage inflammatory protein-1β (MIP-1β) is another chemokine that recruits osteoclast progenitors. Baseline levels for GCF MIP-1β levels prior to surgery were low (Figure 15; 4±1 pg for site A and 3±0.5 pg for site B) which remained low
around teeth at both sites A and B throughout the study period. After a week of healing period, PICF MIP-1β levels were 2.8 fold and 2.2 fold higher than adjacent GCF MIP-1β levels at sites A and B, respectively (14±2 pg for site A and 11±3 pg for site B). The difference between GCF and PICF MIP-1β levels was statistically significant for week 1 only at site A (P=0.02). These levels decreased back to baseline levels by week 4 at both sites. Within group difference between week 1 and week 4 was statistically significant only for implant group at site A (Figure 15; P=0.03).

Vascular endothelial growth factor (VEGF) is a growth factor responsible for angiogenesis and plays a role in regulation of bone remodeling. Expression of VEGF in GCF did not vary significantly between sites A and B prior to surgery, nor at following visits. Level of GCF VEGF was constant throughout the healing period (Figure 16). However, following one week of healing approximately 1.5 fold increase in PICF VEGF levels was noted at both sites A and B, with no significant differences between sites (30±5 pg for PICF VEGF levels at site A and 24±2 pg for PICF VEGF levels at site B; P>0.05). These levels decreased back to baseline levels by week 4 (Figure 16; 17±2 pg for site A and 17±2pg for site B). Within group differences between week 1 and week 4 were not statistically significant following P value adjustment for multiple comparisons (P>0.05).

**Correlations between clinical parameters and local cytokines**

The clinical parameters used to evaluate implant sites were analyzed to determine any correlation to each other at week 1 and week 4 of healing (Table 3). Statistically significant correlation was noted between PI and PD (r=0.356; P=0.022), PI and GI
(r=0.466; P=0.002) and GI and PD (r=0.366; P=0.017) at week 1. Similar correlation was present at week 4 between PI and PD (r=0.349; P=0.022) and GI and PI (r=0.508; P=0.001). In addition, PI was also correlated with PICF (r=0.353; P=0.025) at week 4.

Cytokine correlation tables for week 1 and week 4 were prepared for a total of eight cytokines (Table 4 and Table 5). These cytokines were chosen due to differential expression observed during the study period. More statistically significant correlations among different cytokine pairs were noted for week 1 (20 pairs) compared to week 4 (12 pairs).

Tables 6 and 7 present possible correlations between clinical parameters and cytokine levels at week 1 and week 4 for only peri-implant sites. At week 1, the only statistically significant correlations were detected between PICF volume and IP-10 (r=0.505; P=0.02) and, PICF volume and IL-1β (r=0.458; P=0.04). While at week 4, there was statistically significant correlation between PD and TNF-α (r=0.405; P=0.026).
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**Figure 1 – Study Design**

The study design was a prospective observational trial with a four-week follow-up period.

PI = Plaque Index

GI = Gingival Index

PD = Probing Depth

GCF = Gingival Crevicular Fluid

PICF = Peri-implant Crevicular Fluid

B/L = Buccal/Lingual
Figure 2 – Site A and Site B baseline comparisons for Surgical parameters; p>0.05

Flap = Buccal flap thickness measured with a caliper

Bone = Buccal bone thickness measured with a caliper

KT = Buccal keratinized tissue width measured with a periodontal probe
Figure 3 – Changes in Gingival and Peri-implant Probing Depths During Healing.

* $P \leq 0.03$

** $P=0.002$

BL = Baseline

WKS = Week(s)
**Figure 4** – Plaque accumulation at surgical sites compared to full mouth plaque accumulation; \( p > 0.05 \) (between and within group comparison)

PI = Plaque Index

BL = Baseline

WKS = Week(s)

MOUTH = Plaque accumulation recorded from Ramfjord teeth representing general plaque accumulation for whole mouth
**Figure 5** – Gingival Index Records During Healing Representing Soft Tissue Characteristics; p>0.05 (between and within group comparisons)

GI = Gingival Index

BL = Baseline

WKS = Week(s)

MOUTH = Plaque accumulation recorded from Ramfjord teeth representing general plaque accumulation for whole mouth
Figure 6 – Resonance Frequency Analysis (RFA) Representing Implant Stability;

* P=0.03 (difference between sites for baseline ISQ values)

ISQ = Implant Stability Quotient
BL = Baseline
WKS = Week(s)
**Figure 7** – Gingival and Peri-implant Crevicular Fluid Total Volume. Crevicular fluid was collected by inserting a sterile paper strip into sulcus for 20 seconds. Data is presented as pooled volume for tooth and implant sites.

* *p=0.002

** *p=0.05

BL = Baseline

WKS = Weeks(s)
Figure 8 – GCF and PICF IL-1β levels for two surgical sites; p>0.05 (between and within group comparisons). Data is presented as total amount of protein in crevicular fluid sample obtained within 20 seconds.

BL = Baseline

WKS = Week(s)
Figure 9 – GCF and PICF TNF-α levels for two surgical sites; $p>0.05$ (between and within group comparisons). Data is presented as total amount of protein in crevicular fluid sample obtained within 20 seconds.

BL = Baseline

WKS = Week(s)
Figure 10 – GCF and PICF IL-6 levels for two surgical sites. Data is presented as total amount of protein in crevicular fluid sample obtained within 20 seconds.

*p=0.02

**p=0.002

BL = Baseline

WKS = Week(s)
Figure 11 – GCF and PICF IL-1ra levels for two surgical sites. Data is presented as total amount of protein in crevicular fluid sample obtained within 20 seconds.

*p=0.04

**p=0.002

BL = Baseline

WKS = Week(s)
Figure 12 – GCF and PICF IL-8 levels for two surgical sites; p>0.05 (between and within group comparisons). Data is presented as total amount of protein in crevicular fluid sample obtained within 20 seconds.

BL = Baseline

WKS = Week(s)
Figure 13 – GCF and PICF G-CSF levels for two surgical sites; p>0.05 (between and within group comparisons). Data is presented as total amount of protein in crevicular fluid sample obtained within 20 seconds.

BL = Baseline

WKS = Week(s)
**Figure 14** – GCF and PICF IP-10 levels for two surgical sites; p>0.05 (between and within group comparisons). Data is presented as total amount of protein in crevicular fluid sample obtained within 20 seconds.

BL = Baseline

WKS = Week(s)
Figure 15 – GCF and PICF MIP-1β levels for two surgical sites. Data is presented as total amount of protein in crevicular fluid sample obtained within 20 seconds.

*\( p=0.03 \)

**\( p=0.02 \)

BL = Baseline

WKS = Week(s)
Figure 16 – GCF and PICF VEGF levels for two surgical sites; p>0.05 (between and within group comparisons). Data is presented as total amount of protein in crevicular fluid sample obtained within 20 seconds.

BL = Baseline

WKS = Week(s)
IL-7 and IL-12 are not included due to zero dilution levels. Cytokines/growth factors marked in red are presented as figures individually.
### WEEK 1

<table>
<thead>
<tr>
<th></th>
<th>PD</th>
<th>PI</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>P=0.022, r=0.356</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>P=0.017, r=0.366</td>
<td>P=0.002, r=0.466</td>
<td></td>
</tr>
<tr>
<td>PICF</td>
<td>P=0.297, r=0.190</td>
<td>P=0.911, r=-0.021</td>
<td>P=0.959, r=0.009</td>
</tr>
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</table>

### WEEK 4

<table>
<thead>
<tr>
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<th>ISQ</th>
<th>PD</th>
<th>PI</th>
<th>PICF</th>
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<tbody>
<tr>
<td>PD</td>
<td>P=0.358, r=-0.149</td>
<td>P=0.022, r=0.349</td>
<td>P=0.002, r=0.353</td>
<td>P=0.07, r=0.286</td>
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<tr>
<td>PI</td>
<td>P=0.185, r=0.217</td>
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<td></td>
<td>P=0.025, r=0.353</td>
</tr>
<tr>
<td>GI</td>
<td>P=0.394, r=0.138</td>
<td>P=0.096, r=0.254</td>
<td>P=0.001, r=0.508</td>
<td>P=0.675, r=0.067</td>
</tr>
</tbody>
</table>

**TABLE 3 - CORRELATION TABLES FOR IMPLANT CLINICAL PARAMETERS AT WEEK 1 AND WEEK 4**
### Table 4 - CORRELATION TABLE FOR LOCAL CYTOKINES AT WEEK 1

<table>
<thead>
<tr>
<th>WEEK</th>
<th>IP-10</th>
<th>IL-8</th>
<th>IL-6</th>
<th>IL-1ra</th>
<th>IL-1β</th>
<th>MIP-1β</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>P=0.008 R=0.519</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>P=0.085 R=0.331</td>
<td>P=0.017 R=0.475</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IL-1ra</td>
<td>P=0.04 R=0.390</td>
<td>P=0.119 R=0.320</td>
<td>P=0.214 r=-0.242</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>P≤0.001 R=0.627</td>
<td>P≤0.001 R=0.664</td>
<td>P≤0.001 R=0.664</td>
<td>r=0.1</td>
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<td></td>
</tr>
<tr>
<td>MIP-1β</td>
<td>P≤0.001 R=0.659</td>
<td>P≤0.001 R=0.650</td>
<td>P=0.008 R=0.491</td>
<td>P=0.186 R=0.257</td>
<td>P≤0.001 R=0.751</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>P≤0.001 R=0.696</td>
<td>P=0.01 R=0.508</td>
<td>P=0.065 R=0.353</td>
<td>P=0.288 R=0.208</td>
<td>P≤0.001 R=0.657</td>
<td>P≤0.001 R=0.789</td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>P≤0.001 R=0.647</td>
<td>P≤0.001 R=0.822</td>
<td>P=0.001 R=0.609</td>
<td>P=0.667 R=0.085</td>
<td>P≤0.001 R=0.854</td>
<td>P≤0.001 R=659</td>
<td>P≤0.001 R=0.581</td>
</tr>
</tbody>
</table>

### Table 5 - CORRELATION TABLE FOR LOCAL CYTOKINES AT WEEK 4

<table>
<thead>
<tr>
<th>WEEK</th>
<th>IP-10</th>
<th>IL-1b</th>
<th>IL-1ra</th>
<th>IL-6</th>
<th>IL-8</th>
<th>TNF-α</th>
<th>MIP-1β</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>P≤0.001 R=0.601</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1ra</td>
<td>P=0.04 R=0.379</td>
<td>P=0.238 r=0.222</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>P=0.159 R=0.264</td>
<td>P=0.526 r=0.124</td>
<td>P=0.502 r=0.127</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>P≤0.001 R=0.638</td>
<td>P≤0.001 R=0.755</td>
<td>P=0.965 R=0.009</td>
<td>P=0.143 r=0.279</td>
<td></td>
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</tr>
<tr>
<td>TNF-α</td>
<td>P=0.229 R=0.226</td>
<td>P=0.428 r=0.15</td>
<td>P=0.585 r=0.104</td>
<td>P=0.003 r=0.524</td>
<td>P=0.167 r=0.264</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIP-1β</td>
<td>P=0.01 R=0.443</td>
<td>P=0.008 r=0.474</td>
<td>P=0.505 r=0.127</td>
<td>P=0.292 r=0.199</td>
<td>P≤0.001 r=0.689</td>
<td>P=0.901 r=0.024</td>
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<tr>
<td>VEGF</td>
<td>P=0.04 R=0.386</td>
<td>P≤0.001 R=0.770</td>
<td>P=0.122 r=0.289</td>
<td>P=0.546 r=0.115</td>
<td>P=0.009 r=0.478</td>
<td>P=0.803 r=0.048</td>
<td>P=0.045 R=0.369</td>
</tr>
</tbody>
</table>
### TABLE 6 - CORRELATION TABLE BETWEEN IMPLANT CLINICAL PARAMETERS AND LOCAL CYTOKINES AT WEEK 1

<table>
<thead>
<tr>
<th>Week</th>
<th>PICF</th>
<th>PD</th>
<th>PI</th>
<th>GI</th>
</tr>
</thead>
</table>
| IP-10 | P=0.02  
 r=0.505 | P=0.816  
 r=0.047 | P=0.642  
 r=0.094 | P=0.358  
 r=0.181 |
| IL-8 | P=0.132  
 r=0.358 | P=0.347  
 r=0.201 | P=0.761  
 r=0.066 | P=0.506  
 r=0.133 |
| IL-6 | P=0.051  
 r=0.431 | P=0.429  
 r=0.159 | P=0.854  
 r=0.037 | P=0.354  
 r=0.182 |
| IL-1ra | P=0.427  
 r=0.183 | P=0.665  
 r=0.087 | P=0.550  
 r=0.120 | P=0.846  
 r=0.038 |
| IL-1β | P=0.04  
 r=0.458 | P=0.394  
 r=0.171 | P=0.502  
 r=0.135 | P=0.450  
 r=0.149 |
| MIP-1β | P=0.154  
 r=0.322 | P=0.141  
 r=0.291 | P=0.160  
 r=0.278 | P=0.209  
 r=0.245 |
| TNFα | P=0.094  
 r=0.374 | P=0.367  
 r=0.181 | P=0.190  
 r=0.260 | P=0.117  
 r=0.303 |
| VEGF | P=0.129  
 r=0.343 | P=0.593  
 r=0.108 | P=0.887  
 r=0.029 | P=0.582  
 r=0.109 |

### TABLE 7 - CORRELATION TABLE BETWEEN IMPLANT CLINICAL PARAMETERS AND LOCAL CYTOKINES AT WEEK 4

<table>
<thead>
<tr>
<th>Week</th>
<th>PICF</th>
<th>PD</th>
<th>PI</th>
<th>GI</th>
<th>ISQ</th>
</tr>
</thead>
</table>
| IP-10 | P=0.411  
 r=0.165 | P=0.9  
 r=0.024 | P=0.844  
 r=0.038 | P=0.757  
 r=0.06 | P=0.725  
 r=0.073 |
| IL-8 | P=0.305  
 r=0.209 | P=0.469  
 r=0.140 | P=0.809  
 r=0.048 | P=0.253  
 r=0.219 | P=0.464  
 r=0.150 |
| IL-6 | P=0.238  
 r=0.235 | P=0.655  
 r=0.085 | P=0.167  
 r=0.264 | P=0.341  
 r=0.180 | P=0.956  
 r=0.011 |
| IL-1ra | P=0.966  
 r=0.009 | P=0.568  
 r=0.108 | P=0.122  
 r=0.294 | P=0.438  
 r=0.147 | P=0.462  
 r=0.151 |
| IL-1β | P=0.402  
 r=0.168 | P=0.411  
 r=0.156 | P=0.291  
 r=0.203 | P=0.990  
 r=0.002 | P=0.472  
 r=0.148 |
| MIP-1β | P=0.230  
 r=0.239 | P=0.487  
 r=0.132 | P=0.795  
 r=0.051 | P=0.839  
 r=0.039 | P=0.312  
 r=0.206 |
| TNFα | P=0.528  
 r=0.127 | P=0.026  
 r=0.405 | P=0.336  
 r=0.185 | P=0.075  
 r=0.330 | P=0.552  
 r=0.122 |
| VEGF | P=0.870  
 r=0.033 | P=0.476  
 r=0.135 | P=0.083  
 r=0.328 | P=0.778  
 r=0.054 | P=0.329  
 r=0.199 |
CHAPTER IV

DISCUSSION

This study was designed to determine site-specific characteristics of peri-implant wound healing by creating two similar wounds simultaneously within the same oral cavity. We previously reported that it is possible to detect clinical and biological signs of acute trauma caused by surgery. This study aimed to reproduce the same wound model and investigate whether it is possible to differentiate two similar wounds within the same oral cavity by studying their site-specific clinical and biological characteristics. The results show that although several of baseline surgical parameters are very similar between two sites, some of the clinical parameters such as probing depth, ISQ values, and crevicular fluid volume show significant differences at two wound sites. Similarly, some of the local pro-inflammatory cytokines like IL-6, MIP-1β, and IL-1ra are differentially expressed at two surgical sites.

Early failure rate of this short-term study was 18.5% (4 out of total 22 patients). Implant failure rate due to early wound healing complications is not well documented in the literature. However, a general failure rate of 5-10% is reported with long-term follow-up studies\textsuperscript{92, 93} with mandible presenting less failure rate compared to maxilla.\textsuperscript{94} Previous two large scale peri-implant wound healing studies conducted by our research team reported early failure rates of 7% (2 out of total 30 subjects; 80% of the cases being a mandibular site)\textsuperscript{95} and 13% (5 out of 40 subjects; 100% of the cases being a mandibular
site) occurring within the first couple of weeks of healing. In the current study, 45% of the sites were localized at maxillary posterior quadrants with the remainder in posterior mandibular. Of the failed cases in this study, two implants were in the mandible, while two were in the maxilla. Thus, anatomical location was not a factor affecting higher early failure rate detected in the current study.

Age and gender of subjects can affect healing outcome. Impairment in bone healing was correlated with increasing age, as well as in women than men. However, Venza et al. (2009) reported that age and gender did not affect clinical parameters (GI, PI, PD, CAL) or bone loss around dental implants after healing. Current study compared healing of two implant-related wounds within a patient. Therefore, age and gender of subject did not play a major factor in variation of wound healing response between two sites. Nevertheless, gender distribution was similar between female and male. Age distribution among two genders was also similar (55±3 yrs [56 (29-70) yrs] for female and 54±5 yrs [52 (31-74) yrs] for male). Although distribution of tooth types between sites A and B were equal, slightly higher number of sites A were located in maxilla than sites B. Assignment for surgical sites A and B was retrospectively performed and distribution of anatomical location and tooth type was similar between these two sites.

Observation time for the current study was 1 week and 4 weeks following implant placement surgery. Our previous work showed that major changes within clinical parameters and local cytokine levels in relation to early wound healing occur during first three weeks of healing. Our research group and others also reported that clinical soft tissue maturity after implant placement surgery can be established as early as 4 weeks.
Therefore, week 1 and 4 were designated as post-operative exam times to evaluate the level of healing and related parameters in the surgical sites.

Probing depth, a commonly used periodontal clinical parameter, is greater in implants than teeth. This may be due to less resistance to probing around implants than in gingiva. Probing depth measurements are highly reproducible and specific with low sensitivity. Change in probing depth is considered as a reliable and sensitive marker for bone level change around the implant. Some researchers reported that frequent probing during healing period is reported to increase mean pockets depths around implants, as well as disrupt the epithelial and connective tissue attachments. Another study reported that probing can cause separation of the junctional epithelium and the implant surface. However, histological data obtained from animal model concluded that five days after mechanical separation, a new epithelial attachment is observed, creating a complete regeneration of the implant mucosal seal. Therefore, the effect of probing at early stages of healing is not detrimental on the soft tissue seal. The results of this study show that peri-implant probing depths are statistically deeper than adjacent gingival probing depths at both surgical sites.

Health of the peri-implant tissues is typically monitored through visual inspection, assessment of plaque score, and examination for any inflammatory signs, including bleeding upon probing. Other modalities include radiographic examination, analysis of crevicular fluids and bacteria. Stability of the implant can also be monitored by clinical mobility, indicating failed implant, or Resonance frequency analysis (RFA). When plaque scores of patients with healthy implants are compared with patients with failing implants, no significant differences are noted. However, Jepsen et al (1996) reported
high predictive negative value of gingival scores in identifying sites with progressive attachment loss around implants. This study reported a well-controlled PI and GI during observation time. Implant placement surgery is indicated only for those subjects with healthy periodontium and well-controlled oral hygiene. Thus, baseline PI was low and remained low throughout the study. All subjects were prescribed chlorhexidine 0.12% mouth rinse for the first week, which helps control plaque accumulation.\textsuperscript{102, 103} Similarly, clinical protocol that requires 1 week and 4 weeks post-operative appointments helps clinician to minimize plaque formation at the healing wound site. An increase in GI was observed at week 1 at both surgical sites, although not statistically significant. This increase is expected as a result of surgical trauma and the effect of fresh wound diminishes by week 4.

RFA is a helpful tool in monitoring the stability of the implant. It has high reproducibility/repeatability.\textsuperscript{16} It correlates positively with implant insertion torque.\textsuperscript{48, 49} Stable implants are reported to range between 55 to 84 during healing period.\textsuperscript{43} Another study reported implant stability could be reliably determined for implants with ISQ greater than or equal to 47.\textsuperscript{104} It correlates negatively with bone loss;\textsuperscript{46} however, there is no predictive value for implant success. A single ISQ reading is of limited value due to insensitivity of RFA to predict unstable implants. Failing implants show significant drop from baseline values.\textsuperscript{40, 105} However, a decrease in ISQ reading does not always warrant a failing implant. It has been reported that RFA is influenced by implant length.\textsuperscript{17, 42, 48, 49} Diameter, however, may not affect the RFA value at implant insertion.\textsuperscript{43, 106} Guler et al. (2011)\textsuperscript{47} reported that the ISQ value did not differ significantly between 4.8mm and 4.1mm diameter implants during healing. Bone density \textsuperscript{42, 50, 106} and location in the jaw\textsuperscript{49}
significantly affects RFA values. Mandibular implants have higher ISQ than maxillary implants\textsuperscript{41} with posterior mandible with the highest ISQ\textsuperscript{127} and the lowest in posterior maxilla.\textsuperscript{47} While RFA correlates to cortical bone thickness,\textsuperscript{46, 106} coronal cortical plate thickness does not correlate at all with RFA.\textsuperscript{106} Lastly, some studies report that males show higher RFA values than females.\textsuperscript{47, 49} The results of this study show a statistically significant difference in baseline ISQ readings between surgical site A and site B, with site A having lower ISQ values. This may be due to more implants located in the maxilla at site A compared to site B. Implant diameter and length were unlikely factors in ISQ variation, as they were similar between groups (4.2±0.1mm and 4.3±0.1mm implant diameters for sites A and B, respectively; 10.7±0.2mm and 10.6±0.2mm implant lengths for sites A and B, respectively). Eleven of sites A and nine of sites B had been grafted with bone graft (Freeze Dried Bone Allograft) prior to implant placement. As shown by previous research studies, RFA measured immediately after implant placement and at one month is not affected by history of previous grafting procedure in both mandible and maxilla.\textsuperscript{107, 108} Data from our research group previously confirmed these findings.\textsuperscript{95}

A decrease in ISQ values during first two weeks is expected due to initial bone resorption and callus formation around dental implant.\textsuperscript{40, 43, 45} This is reported to be more significant around week 3 and a complete recovery is seen by week 6.\textsuperscript{41} The results of this study showed similar recovery by week 4 at site A. However, a decrease in ISQ values from baseline was observed at week 4 for site B, although not significant. This was due to two implants with ISQ values decreasing from 68 to 48 ISQ in one case, and from 72 to 37 ISQ in another case. These numbers were not considered as outliers and were included into data analysis for characterization of site-specific wound healing.
Crevicular fluid volume has been reported as higher around functioning implants than GCF of dentition in health. The flow is positively correlated with clinical parameters. Some studies reported positive correlation between PICF volume and plaque accumulation and it is generally accepted that with increasing level of disease, the crevicular fluid volume increases. It is also expected that crevicular fluid volume should increase with any type of surgical trauma. The previous work from our research group reported an increase in PICF and surgically manipulated adjacent GCF volume at week 1 similar to results of this study for wound site A. However, the current study shows that there is a statistically significant difference between PICF collected at wound site A and B during week 1. Thus, host response to acute trauma created by introduction of an implant device may have site-specific differences.

Unlike serum samples, CF sampling collects the entire volume of fluid at the site. Therefore, a method to collect to a standardized time was used for this study. Lamster et al. (1988) reported that when data are expressed as total enzyme amount per standardized time period, correlation to clinical parameters were more sensitive than data presentation as concentration. Other studies also agree. Therefore, our data are presented as total amount of protein rather than as concentration.

Cytokine expression that parallels increase/decrease patterns observed in PICF/GCF volume collected at each observation time was anticipated. In this study, several of the investigated pro-inflammatory cytokines and growth factors followed similar patterns as PICF/GCF volume changes. Few of these cytokines reached statistically significant levels following adjustment of P values for multiple comparisons. Cytokines IL-1β, TNF-α, IL-8, G-CSF, MIP-1β and VEGF were highly expressed at
implant sites and moderately expressed at adjacent surgically manipulated tooth sites at week 1. IL-6 presented statistically higher levels of expression in PICF compared to GCF at site B. There was a tendency (P=0.06) for higher levels of IL-6 expression at PICF than GCF at site A. Also, MIP-1β had statistically higher expression levels in PICF compared to GCF at site A only (P=0.03). Other cytokines such as IL-1ra and IP-10 did not follow PICF/GCF volume fluctuation pattern. These cytokines were expressed at lower levels within PICF compared to GCF at both sites, with statistically significance found only for IL-ra differential expression. The differential expression of these cytokines may be due to history of trauma from extraction, guided bone regeneration/socket preservation, and/or differing surgical trauma related to the implant placement between the sites.

In this study, expression level of IL-1β at surgically manipulated tooth remained stable during healing time from baseline at both sites A and B, which did not parallel the increasing levels of GCF volume at week 1. The levels at implants at week 1 post-op was slightly higher (p>0.05) compared to levels at teeth, which is similar to a previous study. This increased in PICF, then, decreased by week 4. The levels detected in our study were comparable to our previous findings, except expression at implant site A in this study was higher after 1 week of healing, showing differential site-specific response. The values of our studies correlated positively with PICF volume at week 1 only and did not correlate with GI at all periods, unlike previous reports, which may be due to different laboratory protein detection method usage and presentation of data in concentration in the aforementioned studies. Although cytokines, such as IL-1β, increase in expression especially around implants 1 week after surgery, no statistical significance were found due to wide variations in healing response, causing large standard error.
The total amount of TNF-α was relatively low and stable throughout the study period in adjacent teeth. However, it was slightly increased (p>0.05) during week 1 at implant sites A and B, which decreased to GCF levels by week 4. The levels at implant sites A during week 1 tended to be higher than sites B, although not statistically significant. The TNF-α levels expressed in PICF of this study were higher than our previous report. This may be due to usage of single implant system in this study (e.g. less cofactors), unlike the previous study that reported a mean from four implant systems. Despite previous studies reporting positive correlation with inflammation, our reports disagreed. There was no correlation to GI; however, there was a correlation between TNF-α and probing depth at week 4 only. Petkovic et al (2010) reported a positive correlation between IL-1β and TNF-α around healthy implants. Such relationship would be expected since TNF-α and IL-1β have similar functions. However, in our study, positive correlation between these two pro-inflammatory cytokines was noted at week 1 only. Therefore, this may support IL-1β and TNF-α as initial response to surgical trauma, which gets suppressed at later time in healing.

This study confirmed that IL-6 plays a major role in modulating acute response to surgical trauma. While the levels are expressed at low levels at all times, even in health around natural dentition and implants, the expression increased at both implants and tooth sites at week 1 post-operatively. Differential expression was noted from PICF to GCF only at sites B (P=0.002). IL-6 levels of our study were very similar to our previous findings, except levels in PICF were slightly higher in our study at week 4.

IL-1ra is an anti-inflammatory cytokines that inhibits bone resorption that is induced by IL-1β. This study showed that IL-ra is expressed at high levels in health. The
level decreases slightly during week 1, while inflammation is the highest, at both implant and tooth sites and increases as the site heals by week 4. However, there was no significant correlation between inflammation and IL-ra level. The expression around implants was significantly lower than teeth levels, congruent to the expression patterns reported in our previous findings.\textsuperscript{1} However, the levels detected in current study was much lower than previous report.\textsuperscript{1} This may be due to smaller population sample in this study, as well as anatomical locations different from previous study. Despite our expectation that IL-1ra and IL-1\(\beta\) are correlated negatively to each other, no correlations were found.

Similar to a previous study,\textsuperscript{70} higher levels of IL-8 were expressed at implants in both sites A and B at week 1, compared to teeth. This level decreases significantly by week 4, back to levels similar to GCF content, which disagreed with a previous report that healthy functioning implants have higher concentration of IL-8 compared to teeth.\textsuperscript{53, 73} This may be due to absence of loading on implants in our study. Our previous study\textsuperscript{1} showed that IL-8 levels actually decreased at both implants and tooth sites by week 3. Therefore, current study confirms fluctuation pattern reported by our previous study. The levels of IL-8 in our study, however, tended to be lower at both teeth and implant sites. Despite previous reports that IL-8 levels increase with level of inflammation,\textsuperscript{56, 74} current study did not find any significant correlation between IL-8 and clinical parameters.

Our study showed differential healing response between sites A and B in MIP-1\(\beta\) expression. Only at site A, MIP-1\(\beta\) expressions around implants were significantly higher at week 1 than teeth levels, which decreased significantly by week 4. Fluctuation patterns and total amount of MIP-1\(\beta\) expression during early healing are similar to our previous
findings.\textsuperscript{1} In addition, a positive correlation between IL-8 and MIP-1β was found (P<0.001 at week 1 and week 4), similar to previous findings entailing mucositis.\textsuperscript{56} This confirms key roles in IL-8 and MIP-1β in attracting neutrophils and monocytes into early healing sites created by surgery.

The levels of pro-inflammatory G-CSF and IP-10 in PICF have not been studied previously. In current study, the levels of G-CSF in GCF were constant during healing time from baseline. However, the total amount of expression in PICF was increased after one week of healing, although no significant differences between sites A and B, or within sites were noted. Expression level of IP-10 is lower at week 1 of healing, with increase in level of inflammation, which decreases with time. This confirms its anti-inflammatory role. No significant differential expression between sites A and B were noted.

Although studies report positive correlation of VEGF level with clinical parameters,\textsuperscript{116,117} our study did not find any significant correlation to PI, GI, PD, or PICF volume. Despite increase in inflammation due to surgical manipulation of the sites, a non-significant increase was noted only at implants at week 1. Levels around surgically manipulated teeth remained stable throughout the study period. This may be due to difference in blood supply to implants compared to natural dentition and suggests VEGF as a primary cytokine inducing angiogenesis in areas with hypoxia.\textsuperscript{117}

Similar to other studies on wound healing, this study reports significant individual variations in host response. Several factors may be involved in these variations. The simplest explanation can be related to wound size and duration of surgery.\textsuperscript{115} Similarly, the severity of the surgical trauma caused at both hard and soft tissue levels, mechanical stress during implant device insertion, and previous scarring at the surgical site should be
In the current study, effects of wound size and location on healing response were controlled by creating similar wounds at two posterior quadrants. Both sites A and B underwent surgery at the same session and by the same clinician. In addition, single type of implant system was used to eliminate different drilling and insertion protocols for various implant systems.

An edentulous site has been surgically manipulated already from the extraction procedure. It has been reported that oral mucosa heals with significantly less scar tissue formation compared to skin. Nevertheless, an edentulous site may differently react to a surgical trauma compared to previously un-treated site. This is important for sites treated with alveolar ridge preservation or guided bone regeneration procedures prior to implant placement. Related knowledge is very limited. The current study entails healing response in edentulous sites with history of grafting procedures in approximately half of the cases. The distribution of grafted wound sites is similar between sites A and B. Thus, the influence of previous surgical manipulation on results of this study was minimal.

In Summary, very few studies exist to understand the local cytokines related to healing process after one-stage implant placement. Detection of irregularity in expression of these inflammatory cytokines can help predict post-operative complications and/or determine clinical outcome at early healing period. Although our study showed wide variation in healing response between and within individuals, general patterns of cytokine, chemokine, and growth factor expressions are noted in crevicular fluids from surgical sites during early healing:
• IL-1β, TNF-α, G-CSF, IL-8, MIP-1β, VEGF have similar expression patterns. Expression around surgically manipulated adjacent teeth is relatively low and does not change during the first 4 weeks of healing. Expression is higher during week 1 around implants, which decreases to levels similar to those in GCF by week 4.

• IL-6 increases at 1 week post-operatively at both implant and teeth sites, which decreases by week 4. Expression levels are higher around implants than teeth at all time points during early healing period.

• IL-1ra is expressed at high, steady levels around adjacent teeth during the first 4 weeks of healing. The levels around implants are significantly lower than GCF levels. Pattern of IP-10 is similar to IL-1ra. However, the expression level is much lower.

Although cytokines follow these general patterns, differential expression between sites A and B were detected for crevicular fluid volume, IL-6, IL-1ra, and MIP-1β. Pro-inflammatory cytokine, such as IL-1β, TNF-α, IL-8, G-CSF, and growth factor, such as VEGF, tended to be expressed in higher levels in sites A than B, although not significant.

Thus, it was possible to detect site-specific characteristics of acute surgical trauma in two simultaneously created wounds within a subject. Although no site-specific differences were noted in clinical parameters, except ISQ and CF volume, differences in peri-implant crevicular fluid contents were noted at protein level during early healing period.
With better understanding of wound healing process around dental implants and site-specific differences at clinical and protein levels, future studies should aim to study the expressions of these markers at soft tissue level.
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