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PERI-IMPLANT VERSUS PERIODONTAL WOUND HEALING

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Abstract

Aim—Peri-implant gingival healing following one-stage implant placement was investigated and compared to periodontal healing.

Methods—Healing at surgical sites (implant (I) and adjacent teeth (T+)) was compared to non-operated tooth (T-) in non-smokers receiving one-stage implant. Periodontal Indices (PI, GI) were recorded at surgery and up to 12 weeks postoperatively. Peri-implant (PICF) and gingival crevicular (GCF) fluids were analyzed for cytokines, collagenases and inhibitors. Data was analyzed by linear mixed model regression analysis and repeated measures ANOVA.

Results—40 patients (22 female; 21-74 yrs old) completed the study. Surgical site GI, increased at week 1, decreased significantly during early healing (weeks 1-3; p=0.0003) and continually decreased during late healing (weeks 6-12) for I (p<0.01). PICF volume decreased 3-fold by week 12 (p=0.0003). IL-6, IL-8, MIP-1β, and TIMP-1 levels significantly increased at surgical sites at week one, significantly decreasing thereafter (P<0.016). Week one IL-6, IL-8 and MIP-1β levels were ~3-fold higher, and TIMP-1 levels 63% higher, at I compared to T+ (p=0.001).

Conclusion—Peri-implant gingival healing, as determined by crevicular fluid molecular composition, differs from periodontal healing. The observed differences suggest that peri-implant tissues, compared to periodontal tissues, represent a higher pro-inflammatory state.

Keywords

dental implant; periodontal surgery; crevicular fluid; gingiva; wound healing; cytokines

INTRODUCTION

Peri-implant soft tissue healing is described as gingival seal formation (Berglundh *et al.*, 2007). The implant-gingiva interface consists of sulcular and junctional epithelium and underlying connective tissue (Berglundh *et al.*, 2007). Epithelial attachment occurs as early as week 1-2 postoperatively and epithelial barrier matures within 6-8 weeks (Berglundh *et al.*, 2007). In contrast to teeth, connective tissue fibers do not penetrate the implant and are organized parallel to the implant surface, generating a cuff (Berglundh *et al.*, 1996). Because of the lack of periodontal ligament (and its vascular anastomoses to periodontium and

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alveolar bone), supraperiosteal blood vessels consist the sole blood supply of peri-implant gingiva (Berglundh *et al.*, 1994). Such anatomic differences have suggested that peri-implant gingiva may have impaired defenses compared to gingiva around teeth. Recent studies comparing dental implants and teeth without clinical signs of inflammation reported that peri-implant crevicular fluid (PICF) pro-inflammatory cytokine levels were much higher than corresponding gingival crevicular fluid levels (GCF) (Nowzari et al., 2008; 2010). These findings suggest that peri-implant gingiva may be characterized by a higher pro-inflammatory state under apparent homeostatic conditions (clinically healthy tissues). However, information on the molecular events occurring during early peri-implant wound healing, i.e., during dynamic tissue conditions, is scarce.

Detection of early peri-implant tissue changes through conventional clinical methods (e.g., probing) is limited. GCF, with its rich content of locally-derived mediators, mimics wound fluid following surgery. Among several molecules, the following have been reported to be involved in the acute inflammatory response to surgical trauma: interleukin-8 (IL-8), a potent chemotactic agent for neutrophils (Okada H & Murakami S., 1998); macrophage inflammatory protein-1 (MIP-1), another chemokine that selectively stimulates monocyte and lymphocyte migration to inflammation sites (Petkovic AB et al., 2010); interleukin-1 (IL-1), a pro-inflammatory polypeptide implicated in a wide range of biological processes including inflammation, tissue breakdown and tissue homeostasis (Tatakis DN 1993); interleukin-6 (IL-6), another pro-inflammatory cytokine associated with elevated neutrophil elastase levels (Baumann H & Gauldie J., 1994); matrix metalloproteinase-8 (MMP-8) and matrix metalloproteinase-9 (MMP-9), the two main collagen degrading enzymes in GCF (Ingman T et al., 1996), are secreted from neutrophils during activation and are responsible for extracellular matrix degradation; metallopeptidase inhibitor 1 (TIMP-1), a major inhibitor of MMPs, is released at higher levels in inflamed gingiva (Nomura T et al., 1998); and vascular endothelial growth factor (VEGF), a critical mediator of angiogenesis (Pathak AP et al., 2008). Modulation of these tissue byproducts plays a major role in soft tissue integrity (Griffiths 2003). The peri-implant wound created by surgical trauma is expected to exhibit an acute phase response that controls tissue damage and infection and stimulates repair; this fast and robust response should be detectable by studying PICF. Implant placement under one-stage protocol allows optimal access to evaluate such early phases of healing in peri-implant tissue and to compare with surgically accessed adjacent teeth in the same individual.

We hypothesized that the wound healing response of peri-implant gingival tissues following implant placement surgery differs from the healing response around surgically manipulated teeth. Thus, the purpose of this study was to assess and compare gingival wound healing, including clinical and biological parameters, around dental implants (following implant placement surgery) and periodontally healthy teeth (following access flap surgery).

MATERIAL AND METHODS

Study population and study design

Fifty-seven patients treatment planned for mandibular posterior sextant one-stage single implant placement were recruited (Graduate Periodontology Clinic, Ohio State University; March 2009-January 2011) for this prospective observational cohort study. Eligibility criteria were: non-smokers, aged 18-75 years; treatment planned to receive single implant on mandibular posterior sextant; implant site bounded by natural teeth; no systemic diseases/conditions affecting periodontal health or healing; no untreated periodontal disease; able and willing to provide informed consent for surgery and study. Exit criteria were: placement changed to two-stage protocol (Implant Stability Quotient (ISQ) ≤45 or concurrent bone grafting needed); voluntary withdrawal; non-compliance with study protocol; no longer

meeting eligibility criteria (development of systemic/oral disease). Institutional Review Board (Ohio State University) approved study protocol and informed consent forms.

With respect to any prior periodontal disease history, comprehensive periodontal examination was completed and periodontal diagnoses and related treatment plan were provided to potential study participants prior to recruitment; only patients who had successfully completed the recommended periodontal therapy were recruited. Following screening/recruitment visit and eligibility verification, surgery was scheduled. Immediately prior to surgery, plaque (PI) and gingival (GI) indices (Loe 1967) were recorded and GCF samples were collected from the two teeth bounding the implant site (T+: two teeth combined as single site) and from a non-operated contralateral tooth (T-). After implant placement, implant primary stability was assessed by resonance frequency analysis (RFA). At weeks 1, 2, 3, 6, 8, and 12, PI, GI measurements and GCF/PICF collection were obtained from T+, implant (I) and T- sites. Implant stability was again assessed at weeks 1, 3, 6, 8, 12 (data reported elsewhere). Therefore, the study included seven visits (surgery; weeks 1, 2, 3, 6, 8, 12).

Surgical and postoperative protocol

Following local anesthesia, sulcular incisions around teeth and crestal incision over edentulous site were performed. After full-thickness flap elevation, osteotomy site was prepared using custom-made surgical template. Screw type, root form implants(Astra Tech, Moindal, Sweden; Straumann USA, LLC, Andover, MA,USA; Zimmer Dental, Carsbal, CA, USA) were placed following manufacturer's recommendations. After implant placement and stability assessment, polished and platform-matched transgingival healing abutment (height=2-4 mm, dependent on tissue thickness, to avoid soft tissue covering the abutment during the early post-operative period) was screwed to implant and flaps were sutured (standard one-stage protocol). Routine post-operative care was not modified for the study. Post-operative instructions included 1-week abstinence from mechanical plaque control (surgical sites), antimicrobial rinse (0.12% chlorhexidine rinse) use and analgesics (prn). Prescription medication use was recorded through patient diaries. Sutures were removed at week 1.

Clinical Data and Sample Collection

A single trained and calibrated examiner obtained clinical parameters and GCF/PICF samples. PI and GI were recorded (six sites/tooth; T+ and T-) immediately prior to surgery and all postoperative visits (weeks 1, 2, 3, 6, 8, 12). Modified PI and GI (Mombelli *et al.*, 1987) were recorded (four sites/implant; I sites) at weeks 1, 2, 3, 6, 8, 12.

For GCF/PICF collection (one strip/site; 4 sites/implant or tooth), sites were isolated (cotton rolls), supragingival plaque removed using probe, and tissues gently air-dried. Paper strips (Periopaper, Proflow Inc. Amityville, NY, USA) were sequentially introduced into sulci until mild resistance was felt, removed after 20 seconds, and collected fluid volume immediately determined by calibrated instrument (Periotron 8000, Ora Flow Inc, Plain View, NY, USA). Contaminated samples (blood, saliva) were discarded. GCF/PICF samples from each tooth/implant were pooled, placed into sterile cryovials kept on ice, and transferred to -80°C for storage.

Following manufacturer's guidelines, a transducer (implant system/diameter specific; Smartpeg™, Straumann USA, LLC, Andover, MA, USA) was hand-torqued into implant body to measure implant stability by RFA (Osstell™, Mentor, Straumann USA, LLC, Andover, MA, USA). Three ISQ measurements/implant were taken from buccal and lingual aspect and averaged.

Sample Preparation and Analysis

Paper strips were separated from waxed portion using sterile scissors and transferred into prepared sterile microtubes. After addition of sterile cold PBS (200 μl/sample; Cellgro® Dulbecco's Phosphate Buffered Saline Mediatech Inc., Manassas, VA, USA) and 15-minute incubation (occasional vortexing) on ice, samples were centrifuged (13,000 g; 10 min). Eluant was collected and centrifugation repeated once to collect additional volume.

Commercially available multiplex bead-based assay kits (human cytokine group I kit, Bioplex™ Cytokine Assay, Bio-Rad Laboratories, Inc., and MMP/TIMP kit, R&D Laboratories LTD., Antrim, Northern Ireland) were used to detect IL-1β, IL-1ra, IL-6, IL-7, IL-8, IL-10, IL-12, Eotaxin, FGF-b, MCP-1, TNF-α, MIP-1β, VEGF, MMP-8, MMP-9, TIMP-1, TIMP-2, TIMP-3 and TIMP-4 in GCF/PICF samples, following manufacturer's instructions. Both protein concentration and total amount per sample are reported; however, statistical analysis is based on total amount.

Statistical analysis

Sample size was determined by a-priori power analysis, to allow detection of 3-fold difference between baseline GCF IL-8 levels and post-operative week 1 GCF/PICF IL-8 levels, at alpha=0.05 with a 80% power, based on previously published data (Khoury et al., 2008). Because of anticipated individual variations in specific cytokine responses during wound healing and the specific study design (inclusion of additional variables and observation time points), the sample size was doubled (n=40), and the recruitment goal was increased (n=57) to account for anticipated attrition (30%).

Data analysis was conducted by expert consultant (VY) using computer software (SAS/STAT® version 9.2; SAS Institute Inc. Cary, North Carolina, USA). Data was analyzed to detect differences during early (weeks 1-3) and late (weeks 6-12) healing. A linear mixed model regression analysis was used for repeated measures fixed and random effects within and between groups. Initially a random effect (intercept and slope) regression analysis was conducted to estimate the slopes of the outcome over continuous time for I, T+ and T- groups. Repeated measure ANOVA was used to make the within group comparisons between week 1 and week 12 and between group comparisons at baseline and at week 12. Sandwich estimator was used to control the correlation due to dependence of the observations among repeated measurements. The variability of the outcome within and between subjects was used to estimate standard error that tests regression coefficients. Bonferroni correction was used to conserve the overall type I error at α=0.05. Secondary analyses compared first to last observation time for I, T+ and T-groups for each outcome.

RESULTS

Study population

Forty patients (54.4±2 yrs old; 55% female) completed the study having received 26 and 14 implants replacing mandibular molars and premolars, respectively. Patient exclusion reasons were: surgery modified to two-stage protocol (n=4); implant stability loss by week 3 (n=6); post-operative complications requiring additional treatment (n=2), and failure to comply with protocol (n=5).

Clinical Parameters

Average preoperative PI was <1.0 for periodontal (T+ and T-) sites (Table 1). Only T+ showed statistically significant (p=0.029) PI differences during early healing. At I, PI decrease during early healing was non-significant (p=0.08) while week 12 PI differed

significantly from week 1 (p=0.0003). Between group comparisons revealed no significant differences.

At surgical (T+ and I) sites, week 1 GI was increased significantly (p=0.01) compared to T+ baseline GI (Table 1). Surgical site GI declined significantly between weeks 1 and 12 (p=0.0003). Between group comparisons showed that non-surgical controls had significantly lower GI than either surgical site (p=0.0003), only during early healing.

GCF/PICF volume changes (Table 1) were based on total volume (pooled 4 strips/site). GCF/PICF volume decreased significantly during early healing for surgical sites (p<0.001). Volume differences between weeks 1 and 12 were also significant for surgical sites (I:p=0.0003; T+:p=0.02). T- GCF volume changes were non-significant throughout. During early healing, T-sites exhibited significantly less volume than either surgical site (I: p=0.0003; T+: p=0.0006).

Biological Parameters

All molecules, except TIMP-3 and TIMP-4, were detectable in GCF/PICF. However, IL-7, IL-10, IL-12, Eotaxin, FGF-b, TNF-α, and MCP-1 were found at low levels throughout, while consistently high IL-1ra levels showed no significant differences within/between sites (data not shown).

IL-8 and MIP-1β were differentially released at surgical sites with higher levels during early wound healing (Figure 1; Table 2). Week one IL-8 was high at surgical sites, decreasing 7-fold at I (p=0.002) and ~3-fold at T+ (p=0.0002) by week 3. IL-8 decrease at I, between weeks 1 and 12, was significant (p=0.0003). Early healing IL-8 levels were significantly different between T- and surgical sites (I, p=0.007; T+, p=0.003). At late healing, slight but significant increase was detected at T+ (p=0.02), without significant difference between T+ and I (p=0.1). At 12 weeks, IL-8 levels at I were lower than either periodontal site (p>0.05).

A sharp increase in MIP-1β levels (Figure 1; Table 2) at I at week one was followed by 2-fold decrease by week 3 (p=0.016). MIP-1β decreases between weeks 1 and 12 were also significant for I (p=0.0003). The decrease observed at T+ was moderate but still significant at early healing (p=0.007). All sites reached baseline values by week 12 with statistically significant difference between I and T+ at late healing (p=0.03).

IL-1β was higher at surgical sites compared to T- even prior to surgery (baseline T+ vs T, p=0.003) (Table 2). The decrease observed at I between weeks 1 and 12 was significant (p=0.0006). Most of this PICF IL-1β decrease occurred during early healing (p=0.003). Comparisons between groups showed that I sites were significantly different from T- site during early (p=0.03) and T+ site during late (p=0.004) healing. During late healing, periodontal site IL-1β increased, becoming significant for T+ by week 12 (p<0.001; vs. week 1).

Surgical manipulation induced significant IL-6 levels at surgical sites at week 1 (Figure 1; Table 2). IL-6 levels decreased significantly (p≤0.0001) for both surgical sites during early healing. IL-6 level decreases between weeks 1 and 12 were also significant (I, p=0.0003; T +, p=0.01). Between group comparisons revealed that I sites had higher IL-6 levels than periodontal sites (T+, p=0.01; T-, p=0.0003) during early healing. Similarly, IL-6 level difference between T+ and T- was significant during early healing (p=0.0003). By week 12, IL-6 levels returned to the low baseline values for all sites.

PICF VEGF levels at week 1 reached baseline GCF levels and were significantly decreased by week 12 (p=0.0001). Fluctuations in VEGF levels from week 1 through 12 were minimal

for periodontal sites. VEGF differences between I and T+ became significant (p=0.001) at 12 weeks due to continuous decrease at I (Table 2).

MMP-8 and MMP-9 were highly expressed in GCF at baseline (Table 3). Surgical manipulation caused significant decreases in GCF MMP-8 levels during early healing (T+, p=0.001; T+ vs T-, p=0.008). PICF MMP-8 and MMP-9 levels reached baseline GCF levels by week 1 and started decreasing, without significant differences over time. The continuous MMP-9 decrease at I resulted in significant differences between I and periodontal sites (p=0.0003) at early healing (Table 3). There were no significant differences between groups for either MMP at late healing.

At week 1, surgical sites had high TIMP-1 levels (Figure 1; Table 3), which decreased significantly by week 3 ($p<0.0001$) and week 12 ($p\leq0.004$). TIMP-1 PICF levels at late healing were significantly lower than T+ GCF levels (p=0.03). TIMP-2 GCF/PICF levels showed similar temporal patterns (Table 3). Surgical site TIMP-2 levels decreased significantly between weeks 1 and 3 (I, $p<0.0001$; T+, p=0.0004), while PICF TIMP-2 levels also decreased significantly between weeks 1 and 12 (p=0.0003). In contrast, T+ showed slight but significant increase during late healing (p=0.01).

DISCUSSION

The purpose of this study was to assess gingival wound healing responses following implant placement, by comparing relevant clinical and biological parameters between implants and teeth. The results indicate that, in the absence of significant differences in clinical parameters between peri-implant and periodontal tissues during early healing phases, crevicular fluid content exhibits differences in the expression of specific pro-inflammatory mediators between implants and teeth during postoperative healing, revealing a more robust response to surgical trauma in peri-implant compared to periodontal tissues. Therefore, these results support the hypothesis that the wound healing response of peri-implant gingival tissues differs from the healing response around surgically manipulated teeth. To our knowledge, this is the first study to compare peri-implant and periodontal wound healing, at either clinical or molecular level.

Plaque accumulation was well controlled throughout the study and gingiva had minimal inflammation. Periodontal and peri-implant surgical site week-1 GI increases were expected (Cattermole and Wade 1978; DeAngelo *et al.*, 2007; Khoury *et al.*, 2008). Reported peri-implant tissue clinical responses to plaque accumulation or loading (Baldi *et al.*, 2009; Salvi *et al.*, 2012) are not relatable to postoperative healing changes.

GCF/PICF strip collection was limited to 20 sec to minimize contamination risk. This methodological difference may explain the lower volume and mediator levels than previously reported in periodontal health (Okada and Murakami 1998). GCF/PICF volume increases observed at surgical sites at week one gradually declined. Early postoperative GCF volume increases are consistent with previous reports (Cattermole and Wade 1978; Kuru *et al.*, 2004; Kuru *et al.*, 2005). However, early postoperative PICF volume changes are reported for the first time.

GCF/PICF content was reported in both concentration and total amount, but statistical analysis was based on total protein. This approach was chosen since sampling crevicular fluid from a wound site, especially at late time points during healing, results in collecting the entire volume of fluid at the specific site (Lamster and Ahlo 2007). In addition, statistical analysis using concentration values revealed identical results in terms of significance (data not shown). IL-6, IL-8, MIP-1β, and TIMP-1 responses appeared to be surgical trauma-specific with differential several fold increases at both peri-implant and periodontal sites.

The sharp, transient GCF/PICF IL-6 increases following surgery, as reported herein, likely represent the acute response of both soft and hard tissues to surgical trauma (Jawa *et al.*, 2011a; Jawa *et al.*,2011b). We previously reported postoperative PICF IL-8 increases (Khoury *et al.*, 2008), but postoperative GCF/PICF MIP-1β levels have not been previously reported. The exaggerated IL-6 and IL-8 early postoperative responses in I sites (vs. T+ sites), mirror the elevated PICF IL-6 and IL-8 levels (vs. GCF levels) around implants (vs. teeth) without clinical signs of inflammation (Nowzari et al., 2008; 2010). Collectively, these findings suggest that peri-implant gingiva may be characterized by a higher pro-inflammatory state, in the absence of concomitant PI or GI increases. This sub-clinical pro-inflammatory status of the peri-implant gingiva likely reflects unique aspects of peri-implant tissue anatomy and physiology, as indicated by the reported differences between periodontal and peri-implant tissues in extracellular matrix composition (Romanos GE et al., 1995), epithelial cell characteristics (Carmichael RP et al., 1991), and immune cell distribution in both health (Horewicz VV et al., 2013) and disease (Berglundh T et al., 2011). To what extent, if any, this higher pro-inflammatory state confers greater protection against bacterial pathogens or increases the susceptibility of peri-implant gingiva to tissue breakdown following bacterial challenge remains to be determined.

Matrix metalloproteinases (MMPs) are primarily responsible for tissue turnover (matrix degradation/remodeling) in physiological and pathological conditions (Siefert and Sarkar 2012). The balance between local catabolic (MMP) and anti-catabolic (TIMP) activities determines tissue degradation and remodeling (Baker and Leaper 2003). Consistent with previous reports on implant placement surgery (Nomura *et al.* 2000), TIMP-1 levels increased significantly during postoperative week one as a response to surgical trauma in general and, gradually decreased; the postoperative TIMP-1 level increase could be important in limiting the acute post-trauma tissue destruction.

The present study is the first one to provide extensive data on GCF molecular changes occurring in healthy periodontal sites following access flap surgery. Kuru *et al.* (2004, 2005) also studied GCF composition during early postoperative healing following surgery in “control” teeth (T+ equivalent) receiving crown lengthening; however, their GCF analysis was limited to TGFβ1 and specific adhesion molecules, thus precluding comparisons to the results reported here.

In the present study, wound size and location were limited to tooth-bounded mandibular posterior single implant sites. The limited wound size and restricted anatomical location minimized variations in extent and severity of surgical trauma; the control of implant positioning by use of a custom surgical stent designed for a screw-retained crown further minimized variation. This clinical model allows control of relevant variables and inclusion of intra-individual periodontal control (positive/negative) sites. Fourteen implant sites had received prior bone grafting for ridge reconstruction (missing tooth) or preservation (at tooth extraction), with grafting performed at least 4 months prior to study surgery and all 14 cases receiving the same treatment (freeze-dried bone allograft plus collagen membrane). Grafting history did not affect either clinical or PICF parameters (data not shown), consistent with earlier reports of previously grafted peri-implant sites having clinical characteristics similar to non-grafted sites (Sjostrom *et al.*, 2005; Degidi *et al.*, 2007).

Three different implant systems were used in the present study, based on case-specific treatment plan; the systems differ in surface characteristics (especially roughness/porosity) and thread morphology. However, all implants, regardless of system, were root form, screw type, and placed at bone level, and all received a smooth (polished) healing abutment of matching diameter. Therefore, the peri-implant soft tissues were always sutured against the same metallic abutment material, regardless of implant system used; thus, any effects on soft

tissue healing potentially stemming from diverse implant system characteristics were minimized, if not eliminated altogether.

Based on patient diaries, 10 of the 40 patients took systemic antibiotics and 30 took analgesics during postoperative days 1-3, with no association between medication intake and clinical or PICF/GCF parameter changes during week one (data not shown). Pre- or post-operative antibiotic or NSAID use apparently has limited effect on PICF biomarkers, early implant failure, or wound healing (Khoury *et al.*, 2008; Alissa *et al.*, 2009; Waasdorp *et al.*, 2010); nevertheless, evidence suggests that pre-operative antibiotics may contribute to a slight decrease in overall implant failure (Sharaf *et al.*, 2011). Post-operative instructions following surgery included soft diet, chlorhexidine mouth rinse (0.12%) use, and abstinence from performing mechanical plaque control at surgery site for one week; this protocol was applied to all patients. This approach has been shown to effectively control postoperative plaque levels and inflammation (Grusovin MG et al., 2010), and the clinical outcomes of the present study (Table 1) corroborate this finding.

The present study results indicate the feasibility of detecting signs of acute surgical trauma at newly placed dental implant sites and surgically manipulated teeth, by analyzing PICF and GCF, respectively. The robust molecular responses specific to surgical trauma are much more pronounced at peri-implant gingiva compared to surgically-accessed adjacent periodontal tissues; these responses likely represent unique aspects of peri-implant tissue anatomy and physiology (e.g., healing processes). Given the reported effects of surgical technique (flapless placement versus flap surgery) on healing peri-implant soft tissue gene expression and inflammation levels (Mueller CK et al., 2011), additional studies are necessary to determine whether the observed differences between teeth and implants can be attributed, at least in part, to the potential differences between routine healing (reformation of severed periodontal attachment existing around teeth) and healing following wounding of previously intact masticatory mucosa (edentulous site) and de novo contact with foreign (smooth metallic) material. The present investigation model could serve as basis for future studies to differentiate physiological from delayed or compromised peri-implant wound healing.

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Clinical Relevance

Scientific Rationale for the Study: The major anatomical differences (blood supply; nature of connective tissue attachment) between gingiva around teeth and implants may impact wound healing and result in differential crevicular fluid content.

Principal Findings: Inflammation- and tissue degradation/remodeling-specific mediators were detected at high levels in both peri-implant and adjacent periodontal surgically manipulated gingiva, demonstrating a robust acute response to surgical trauma. Some mediators were selectively highly released around implants compared to adjacent teeth during early healing.

Practical Implications: The observed molecular differences between teeth and implants likely reflect unique aspects of peri-implant tissue physiology and anatomy. The present experimental model could serve as basis for future studies to differentiate physiological from delayed/compromised peri-implant wound healing.

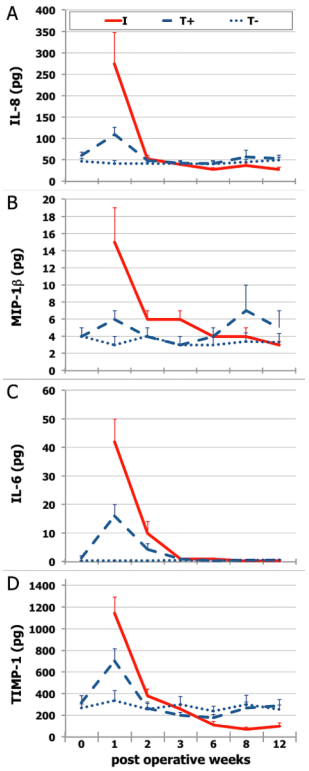

Figure 1. Peri-implant and gingival crevicular fluid biomarkers following implant placement

IL-8 (A), MIP-1β (B), IL-6 (C), and TIMP-1 (D) total amount/site (mean+SE). Each data point represents crevicular fluid samples (4 strips/site; 20 sec/strip) from 40 patients. Each patient was sampled at peri-implant crevice (I), gingival crevice of adjacent tooth (T+), and gingival crevice of contralateral, non-operated tooth (T-). I and T+ were surgical sites (full-thickness flap elevated to place one-stage dental implant). Week 0 (T+ and T-) refers to immediately pre-operative (baseline) samples. Week 1 values differed significantly from week 12 for IL-8 (I, p=0.0003), MIP1-β (I, p=0.0003), IL-6 (I, p=0.0003; T+, p=0.01), and TIMP-1 (I, p=0.0003; T+, p=0.004). At early healing (weeks 1-3), surgical sites differed significantly from T- sites for IL-8, IL-6, and TIMP-1 (adjusted p≤0.05); as did I from T+ for IL-6 (p≤0.05). At late healing (weeks 6-12), I differed from T+ for MIP1-β (p≤0.03). Additional details provided in text.

Table 1

Clinical Parameters During Early and Late Healing

PI: Plaque Index[*]; GI: Gingival Index[*]; CF: Crevicular fluid volume; I: Implant site; T+: Surgically treated tooth: T-: non-treated control tooth.

	mean±se median(range)						
	Baseline	**1 week**	**2 weeks**	**3 weeks**	**6 weeks**	**8 weeks**	**12 weeks**
PI							
I		0.6±0.1 0.5 (0-2)	0.5±0.1 0.4 (0-3)	0.4±0.1 0.3 (0-2)	0.4±0.1 0.35 (0-1)	0.3±0.1 0.2 (0-2)	0.3±0.1 ◇ 0.2 (0-2)
T+	0.8±0.1 0.8 (0.2-3)	0.7±0.1 0.7 (0-3)	0.6±0.1 0.5 (0-2)	0.6±0.1 • 0.5 (0-2.5)	0.7±0.1 0.6 (0.2-1.8)	0.6±0.1 0.5 (0.2-2)	0.7±0.1 0.5 (0-2)
T-	0.7±0.1 0.5 (0-3)	0.4±0.1 0.5 (0-2)	0.4±0.1 0.2 (0-1.5)	0.5±0.1 0.5 (0-1)	0.5±0.1 0.5 (0-2)	0.5±0.1 0.5 (0-2)	0.5±0.1 0.5 (0-1.2)
GI							
I		1.1±0.1 1 (0-3)	0.6±0.1 0.5 (0-2)	0.4±0.1 •* 0.3 (0-2)	0.2±0.1 0 (0-1)	0.1±0.0 0 (0-1)	0.1±0.0 □◇ 0 (0-0.5)
T+	0.6±0.1 0.5 (0-2)	0.9±0.1 1 (0-3)	0.6±0.1 0.5 (0-2)	0.5±0.1 •* 0.5 (0-2)	0.4±0.1 0.2 (0-2)	0.3±0.1 0.2 (0-2)	0.3±0.1 ◇ 0.2 (0-1.2)
T-	0.4±0.1 0.2 (0-1.5)	0.2±0.0 0 (0-1)	0.1±0.0 0 (0-1)	0.2±0.1 0 (0-1.5)	0.2±0.1 0 (0-2)	0.2±0.1 0 (0-1)	0.2±0.1 0 (0-1)
CF volume (µl)							
I		1.8±0.2 1.6(0.2-4.1)	1.2±0.1 0.8(0.3-3.1)	1±0.1 •* 0.8(0.2-4.6)	0.7±0.1 0.6(0.2-2.2)	0.5±0.0 0.5(0.2-1.1)	0.6±0.1 ◇ 0.5(0.02-2)
T+	1.4±0.1 1.1(0.5-3.6)	1.7±0.1 1.5(0.7-4)	1.5±0.1 1.3(0.4-5.3)	1.3±0.1 •* 1(0.4-3.7)	1.4±0.1 1.2(0.5-3.8)	1.1±0.1 1.2(0.3-2.5)	1.3±0.1 ◇ 1.1(0.4-3.7)
T-	1.2±0.1 0.95(0.2-3.6)	1.2±0.1 0.9(0.1-3.7)	1.2±0.1 1.1(0.3-4.3)	1.2±0.1 0.8(0.2-3.3)	0.9±0.1 0.84(0.2-4)	0.9±0.1 0.7(0.2-2.3)	1.1±0.1 0.9(0.2-4)
ISQ							
I	69.5±1.3 68(52-83)	69.2±1.2 69.3 (51-81)		70±1.3 • 73 (51-85)	75±1 75 (62-85)	75±1.1 76 (62-87)	75±1.3 ◇ 77 (54-86)

• Early healing (week 1 through week 3) statistically significant differences

□ Late healing (week 6 through week 12) statistically significant differences

* between groups I vs T-

* between groups T+ vs T-

◇ Statistically significant difference between week 1 and week 12

* PI and GI were obtained from six surfaces from T+ and T- sites while they were obtained from four surfaces from I site.

Table 2

Crevicular fluid IL-8, MIP-1β, IL-1β, IL-6 and VEGF content

I: Implant site; T+: Surgically treated tooth: T-: non-treated control tooth. Each value represents crevicular fluid samples (4 strips/site; 20 sec/strip) from 40 patients.

	PICF/GCF Cytokine Concentration (ng/ml) PICF/GCF Cytokine Total amount (pg)						
	Baseline	**1 week**	**2 weeks**	**3 weeks**	**6 weeks**	**8 weeks**	**12 weeks**
IL-8							
I		168±36 274±74	58±10 52±8	48±7 39±7 •*	46±8 28±4	77±11 37±6	52±8 28±5 ◇
T+	54±9 60±8	67±10 109±17	40±5 49±7	43±8 42±6 •*	34±5 41±7	49±9 57±16	47±6 53±8 □
T-	47±6 47±7	44±7 41±7	48±7 41±4	46±6 41±5	56±10 40±6	64±9 45±6	63±10 49±6
MIP-1β							
I		9±2 15±4	7±1 6±1	6±1 6±1 •	6±1 4±1	7±1 4±1	5±1 3±1 ⊗*◇
T+	4±1 4±1	4±0.4 6±1	3±0.5 4±1	3±1 3.3±1 •	3±1 4±1	6±3 7±3	5±1.4 5±2 □
T-	4±1 4±1	3±0.3 3±1	4±1 4±1	3±1 3±1	4±1 3±1	4±1 3±1	4±0.5 3±1
IL-1β							
I		13±3 19±3	8±1 7±1	12±2 10±2 •*	11±2 8±1.3	14±2 7±1	13±3 7±2 *◇
T+	17±3 18±3	10±1 16±2	13±3 16±4	17±4 15±3	13±2 14±2	20±4 19±3	25±4 26±4 □◇
T-	11±2 9±2	9±2 8.3±2	9.5±2 9±1	9±1 8.2±1	9±2 6.4±1	11±2 8±1	15±4 11±3
IL-6							
I		28±6 42±8	9±3 10±4	1±0.2 1±0.3 •*⊗	1±0.3 0.6±0.3	0.5±0.1 0.2±0.1	1±0.2 0.4±0.1 ◇
T+	1.2±0.6 1.3±1	11±2 16±4	3±1 4±2	0.6±0.2 1±0.3 •*	0.4±0.1 0.4±0.1	0.7±0.1 0.6±0.1	0.8±0.3 0.5±0.1 ◇

	PICF/GCF Cytokine Concentration (ng/ml) PICF/GCF Cytokine Total amount (pg)						
	Baseline	**1 week**	**2 weeks**	**3 weeks**	**6 weeks**	**8 weeks**	**12 weeks**
T-	0.3±0 0.3±0.1	0.4±0.1 0.3±0.1	0.4±0.1 0.3±0.1	0.4±0.1 0.4±0.2	0.5±0.1 0.3±0.1	0.7±0.2 0.4±0.1	0.8±0.3 0.5±0.2
VEGF							
I		25±3 41±7	24.±3 22±3	37±5 31±4	42±6 26±4	68±13 29±4	45±6 ◇ 22±3 ◇
T+	36±5 40±4	26±3 43±6	26±4 32±6	29±4 32±5 •	23±3 32±5	36±5 38±6	43±6 47±6 □
T-	34±6 30±4	28±4 30±5	29±4 27±3	32±6 28±4	38±9 24±3	39±6 29±4	36±5 29±4

• Early healing (week 1 through week 3) statistically significant differences

□ Late healing (week 6 through week 12) statistically significant differences

* between groups I vs T-

* between groups T+ vs T-

⊗ between groups I and T+

◇ Statistically significant difference between week 1 and week 12

Table 3

Crevicular fluid MMP-8, MMP-9, TIMP-1 and TIMP-2 content

I: Implant site; T+: Surgically treated tooth: T-: non-treated control tooth. Each value represents crevicular fluid samples (4 strips/site; 20 sec/strip) from 40 patients.

	PICF/GCF Protein Concentration (μg/ml) PICF/GCF Protein Total amount (ng)††						
	Baseline	**1 week**	**2 weeks**	**3 weeks**	**6 weeks**	**8 weeks**	**12 weeks**
MMP8							
I		19.3±6 27±7	12.4±2 15±4	16.2±3 18±5	24.2±7 19±6	19.3±4 9±2	22±6 14±5
T+	21±3 25±4	16.4±2 28±4	12±1.6 17±2	14.1±2 16±3•*	13.7±2 23±7	21.4±3 23±4	25.1±4 27±5
T-	21±4 20±4	16.2±3 17±4	20.7±4 21±5	18±2.6 18±4	22.9±6 20±6	18.1±3 15±3	25±5 20±4
MMP9							
I		42.2±5 59±6	31.8±4 35±5	37.3±5 34±5•*	45.4±7 34±5	54.8±10 29±5	50±6.3 29±5◇
T+	44±6 52±7	37.2±4 57±5	30.3±4 41±6	35.2±5 40±5•*	33.1±5 39±5	50.8±6 51±6	51.4±8 51±6▱
T-	50±6 45±6	38.7±6 36±6	47.1±6 45±5	50.6±8 44±5	53.8±8 42±6	52.5±7 44±7	59.1±11 45±5
TIMP-1							
I		1.3±4 1142±151	0.5±0.1 380±61	0.3±0.1 256±42•*	0.1±0.0 110±34	0.2±0.1 70±20	0.2±0.1 99±30⊗◇
T+	0.3±0.1 318±64	0.5±0.1 702±114	0.4±0.1 265±59	0.6±0.3 201±24•*	0.2±0.0 180±28	0.3±0.1 270±52	0.4±0.1 291±54▱◇
T-	0.3±0.0 267±40	1.1±0.8 335 ± 93	0.3±0.1 256±53	0.4±98 302±0.73	0.3±0.1 240±43	0.5±0.2 300±83	0.5±0.1 254±40
TIMP-2							
I		2.3±0.3 2756±457	2.2±0.4 1429±221	2±0.4 1559±295•*	2.5±0.5 1562±304	3.8±0.8 1734±289	5±1.5 1495±255◇
T+	2±1 2708±610	1.9±0.3 2440±365	2.2±0.5 1803±317	2.2±0.4 1828±317•*	1.7±0.3 1756±241	2.8±0.5 1904±240	2.8±0.5 2099±221▱

	PICF/GCF Protein Concentration (μg/ml) PICF/GCF Protein Total amount (ng)††						
	Baseline	**1 week**	**2 weeks**	**3 weeks**	**6 weeks**	**8 weeks**	**12 weeks**
T-	2±0.4 1485±243	2.3±0.4 1548±203	2.6±0.6 1714±268	2.6±0.5 1727±278	2.8±0.9 1472±269	4.5± 1.9 1881±316	3.5±0.7 2034±233

††TIMP-1 and TIMP-2 total amount in pg

•Early healing (week 1 through week 3) statistically significant differences

□Late healing (week 6 through week 12) statistically significant differences

*between groups I vs T-

*between groups T+ vs T-

⊗between groups I and T+

◇Statistically significant difference between week 1 and week 12