Molecular Assessment of Human Peri-implant Mucosal Healing at Laser-Modified and Machined Titanium Abutments

Austin Leong, DDS[1]/Ingeborg De Kok, DDS, MS[2]/
Daniela Mendonça, DDS, MSc, PhD[3]/Lyndon F. Cooper, DDS, PhD[4]

Purpose: *To compare, by gene profiling analysis, the molecular events underscoring peri-implant mucosa formation at machined vs laser-microgrooved implant healing abutments.* ***Materials and Methods****: Forty endosseous implants were placed by a one-stage approach in 20 healthy subjects in nonadjacent sites for single-tooth restorations. In a split-mouth design, machined smooth and laser-microgrooved healing abutments were randomly assigned in each subject. Peri-implant mucosa adjacent to healing abutments was harvested by tissue punch biopsy at either 1, 2, 4, or 8 weeks following abutment placement. Total RNA was isolated from the peri-implant transmucosal soft tissues. A whole genome microarray using the Affymetrix Human Gene 2.1 ST Array was performed to describe gene expression profiles in relation to abutment topography and healing time duration. Data analysis was completed using GeneSpring software v.12.6.* ***Results:*** *Differential gene expression was revealed at all time points and among surfaces. Five hundred one genes were differentially expressed (fold change ≥ 2.0) at machined versus laser-modified abutments, and 459 of these were statistically significant ($P \leq .05$). At 1 week, unique expression of IL-24 and MMP1 was observed in tissues from laser-treated surfaces. At 2, 4, and 8 weeks, mRNAs encoding keratins and protective proteins of cornified epithelium were upregulated in tissues from laser-modified abutments. At 4 weeks, upregulation (> 2-fold) of mRNAs encoding proteins associated with collagen fibril formation and function was observed in tissue from laser-modified abutments. In both tissues of machined and laser-modified abutments, mRNAs encoding junctional epithelium–specific proteins, ostogenic ameloblast associated protein (ODAM) and follicular dendritic cell secreted protein (FDCSP) were highly upregulated throughout weeks 2 to 8.* ***Conclusion:*** *Peri-implant abutment mucosal wound healing involves selective differentiation of epithelium and induction of the junctional epithelium. Laser-mediated alterations in abutment topography enhance collagen fibril-associated gene expression and alter epithelium/junctional epithelial gene expression. Clinically, shallower probing depths are measured at laser-mediated versus machined implant abutments.* Int J Oral Maxillofac Implants 2018;33:895–904. doi: 10.11607/jomi.6367

Keywords: *dental implant abutment, junctional epithelium, laser, mucosal integration, titanium*

[1]Assistant Professor, Restorative Dentistry, Maurice H. Kornberg School of Dental Medicine, Philadelphia, Pennsylvania, USA.
[2]Assistant Professor, Department of Prosthodontics, University of North Carolina School of Dentistry, Chapel Hill, North Carolina, USA.
[3]Clinical Assistant Professor, Department of Biologic and Materials Sciences, Division of Prosthodontics, University of Michigan - School of Dentistry, Ann Arbor, Michigan, USA.
[4]Associate Dean for Research, Head, Oral Biology, College of Dentistry, University of Illinois at Chicago, Chicago, Illinois, USA.

Correspondence to: Dr Lyndon F. Cooper, Associate Dean for Research, Head, Oral Biology, College of Dentistry, University of Illinois at Chicago, 801 S. Paulina St., Room 402e, Chicago, IL 60612, USA. Email: cooperlf@uic.edu

The basis for dental implant success is attributed to osseointegration. The osseointegrated implant in function must be connected to a prosthesis of fixed or removable design by means of a transmucosal abutment. The nature of peri-implant abutment/mucosal integration has received relatively sparse attention; the abutment/mucosal integration is defined histologically. Biofilm accumulation and dysbiosis on implant prostheses and implant abutments induce inflammation of the adjacent mucosa, termed peri-implant mucositis, and continued exposure leads to peri-implant osteolysis or peri-implantitis. Given the prevalence of peri-implantitis (at least 10% of implants), understanding the basis for abutment/mucosa integration is a prerequisite to defining strategies to enhance peri-implant mucosal health.

The process of implant abutment/mucosa integration has been characterized at the histologic level.[1] Over an 8-week period following transmucosal placement of an abutment, wound healing with attendant connective tissue and epithelial attachments are formed. Architecturally analogous to the biologic width that forms along natural teeth,[2] a connective tissue attachment and junctional epithelial (JE) attachment are reproducibly formed along implant abutments made of either titanium or zirconia.[3] The connective tissue attachment contains a fibroblastic cellular zone (approximately 40 µm) directly opposing the abutment and an adjacent collagen-rich zone (160 µm). The collagen fibrils are horizontally oriented parallel to the abutment surface. The peri-implant mucosa is physically attached to the abutment through a narrow zone of JE attachment.

One function of mucosal integration at implant abutments is the immunologic surveillance of the local environment. In the adjacent connective tissues opposing the implant-abutment interface, an inflammatory cellular infiltrate exists.[4] Human studies demonstrate that the inflammatory infiltrate is responsive to biofilm and the biomechanical environment.[5,6] Attributed to mucosal integration is the protection to the underlying implant/bone interface from biofilm/inflammation-induced osteolysis.[7,8]

The abutment/peri-implant mucosa interface is readily distinguished from the tooth-tissue interface in several ways. A direct attachment of connective tissue into cementum as Sharpey's fibers has not been observed. At implant abutments, a dense collagenous connective tissue is oriented with the collagen fibers circumferential to the abutment surface.[9] Importantly, the organization of this tissue is not related to the superimposed architecture of the prosthesis or the abutment, and this creates significant challenges in esthetic implant dental therapy.

Efforts to alter the implant-abutment connection target improvement in peri-implant tissue responses. The implant design (eg, platform switching[10]) as well as abutment design are targeted. Both titanium and zirconia abutments are used, although van Brakel and colleagues[11] demonstrated no significant differences in the structure of the inflammatory cellular infiltrate in humans. Cytokine, chemokine, and bone metabolite levels were similar in peri-implant crevicular fluid (PICF) of titanium and zirconia abutments.[12] Acid-etched implant abutment micron topography alteration did not influence the histologic structure of the abutment-tissue interface in comparison to smooth, machined surfaces. Delgado-Ruiz et al compared concave versus wide machined healing abutments, and histologic analysis revealed connective tissue architecture changes that reflected the morphology of the abutments without significant alteration in the direct attachment.[13] These examples of studies that have altered the material, topography, or shape of the abutment have not substantially altered the peri-implant mucosal interface with the dental implant abutment.

More recently, a laser-ablated micron-scale modification that imparts circumferential, isotropic channels onto the titanium abutment surface altered the connective tissue morphology and was associated with a JE terminating at the isotropic micron-scale grooves.[14,15] Given the importance of micron-scale topography on adherent cell behavior, the aim of this study was to explore the impact of laser-mediated isotropic titanium abutment topography on the healing of human peri-implant mucosa by utilizing genome-wide analysis (gene profile analysis) to systematically determine specific gene expression differences present in peri-implant transmucosal soft tissues at machined smooth versus laser-ablated healing abutments. Two specific hypotheses were investigated: *(1)* micron-scale laser-ablated abutment surfaces mediate significant changes in fibroblastic adhesion characterized by alterations in integrin receptor and extracellular matrix protein expression; and *(2)* micron-scale laser-ablated abutment surfaces mediate alterations in the JE component of the peri-implant mucosa characterized by alterations in expression of JE proteins and markers of oral epithelium development. Together or separately, addressing these hypotheses may provide information that is valuable in understanding the process of peri-implant mucosal healing and the influence of micron-scale isotropic topography on this interface. This study represents the first clinical evaluation of the molecular processes of peri-implant tissue formation and the impact of abutment topography on this process.

MATERIALS AND METHODS

Participant Recruitment

Patients requiring at least two single-tooth endosseous implants were recruited and treated under informed consent and protocol approved by the University of North Carolina at Chapel Hill (UNC-CH) Office of Human Ethics (IRB# 12-1415). Twenty consecutive participants were recruited from the clinics of the University of North Carolina School of Dentistry, as well as community-at-large respondents to the School of Dentistry's website. Subjects were equally assigned to four cohorts comprising a de-escalation protocol of tissue integration time periods of 8, 4, 2, or 1 weeks. Consenting participants met additional inclusion criteria: possessing two or more independent sites (edentulous or requiring extraction due to nonrestorability) requiring

implants for single-tooth restoration with natural teeth adjacent (at least unilaterally) to both sites, ages 18 to 75 years, and available for 1-year follow up. Potential participants were excluded for the following reasons: presence of untreated caries or periodontal disease, unable to demonstrate adequate home oral hygiene, smoked tobacco within the past 6 months, ASA Class 3 or greater or immunocompromised, pregnant or planning to be pregnant within 6 months, exhibiting severe bruxism, history of bisphosphonate use, or insufficient bone for implant placement.

Clinical Protocol

Following consent, all participants had preoperative records made/taken including: standardized digital photographs, digital radiographs, and maxillary and mandibular irreversible hydrocolloid impressions (Jeltrate Plus, Dentsply). Radiographic stents were fabricated from diagnostic waxing of restorations at the proposed implant sites, and cone beam computed tomography scans of sites of interest were obtained (Orthophos XG 3D, Sirona, or CS 9000, Carestream). DICOM files were imported into planning software (SimPlant 15b, Materialise), and implant sites were assessed for placement of implants of 3.8 mm/4.6 mm diameter, 9 mm/10.5 mm length (Tapered Internal Mountless, Resorbable Blast Textured [RBT] Implant, BioHorizons). Note that implants without Laser-Lok features were used in this study. For sites requiring extraction, socket preservation was performed with Bio-Oss bovine xenograft (Geistlich Biomaterials) and a collagen plug wound dressing (CollaPlug, Zimmer Dental), followed by 6 months of healing.

A preoperative dose of 2,000 mg amoxicillin (or 600 mg clindamycin for those with penicillin allergy), as well as 800 mg ibuprofen was provided 30 to 60 minutes prior to surgery. Participants were also instructed to rinse for 60 seconds with an antimicrobial mouthrinse (chlorhexidine gluconate 0.12%). Local anesthesia via infiltration was administered using 2% lidocaine with epinephrine 1:100,000 (Xylocaine, Dentsply) and/or 4% articaine with epinephrine (Septocaine, Septodont). Full-thickness flaps were elevated in the proposed implant sites. Implant osteotomies were prepared and implants placed according to the manufacturer's protocol for placement of 3.8- and 4.6-mm implants.

Following implant placement, one machined smooth titanium healing abutment (Narrow Emergence 3 mm height, 3.5 mm/4.5 mm platform, BioHorizons) and one laser-microgrooved healing abutment (Narrow Emergence, Laser-Lok, 3 mm height, 3.5/4.5 mm platform, BioHorizons) were hand tightened onto the respective implants. Flaps were then closed for each abutment using 4-0 chromic gut interrupted sutures. Periapical radiographs were exposed to observe abutment seating and implant position. A 1-week postoperative course of amoxicillin (or clindamycin) was prescribed, as well as 2-week, twice daily chlorhexidine gluconate 0.12% mouthrinse. Hydrocodone/acetaminophen or ibuprofen was prescribed for analgesia, and homecare instructions were provided. A postsurgical follow-up was conducted at 1 week to ensure compliance with homecare instructions, observe soft tissue healing, and manage any potential complications. Additional visits were scheduled as necessary if any complications presented.

Peri-implant Mucosa Tissue Sampling

A soft tissue biopsy was performed for both implant sites of each participant at either the 1-, 2-, 4-, or 8-week time point. Local anesthesia was administered via infiltration using 2% lidocaine with epinephrine 1:100,000 and/or 4% articaine with epinephrine. Tissue punches were used to circumferentially harvest the peri-implant transmucosal tissues from both the machined and laser-modified abutment of each participant. For sites that received implants of 3.8 mm diameter, a 5 mm sterile tissue punch (Salvin Dental Specialties) was used to retrieve the soft tissue. For sites that received implants of 4.6 mm diameter, a 6 mm sterile tissue punch (Salvin Dental Specialties) was used to retrieve the soft tissue. Tissue samples were immediately placed into RNA stabilization reagent (RNAlater, Ambion), and refrigerated for 24 hours. Following the 24 hours, the RNA stabilization reagent was removed and samples were frozen and stored at –80°C for gene expression analysis.

At the 8-week time point for all 20 subjects, probing pocket depths were recorded at six positions (mesial [M], mesiobuccal [MB], mesiolingual [ML], distal [D], distobuccal [DB], and distolingual [DL]) for both the machined and laser-modified abutments. Subsequently, the abutments were removed and conventional impressions made for definitive abutment (Core 3D Centers North America) and crown fabrication. Following final impressions, all implant sites that received laser-ablated healing abutments were replaced with new laser-ablated healing abutments of the same dimension.[14] Two to 3 weeks later, definitive abutments and crowns were delivered. Sites that received laser-ablated healing abutments received laser-ablated custom abutments. Sites that received machined healing abutments received machined custom abutments.

RNA Isolation

Frozen samples were removed and ground with mortar and pestle in liquid nitrogen. Total RNA in the cell lysates was isolated and purified using the miRNeasy Micro Kit (Qiagen) according to the manufacturer's specifications. Total RNA was assessed for quality and

quantity using a bioanalyzer (Aligent) and nanodrop ND-1000 spectrophotometer (Nanodrop), respectively. Samples were then processed and hybridized to the Affymetrix Human Gene 2.1 ST Array (Affymetrix) at the UNC core facility following the manufacturer's recommended protocols and reagents. The Affymetrix Human Gene 2.1 ST Array plate interrogates more than 30,000 annotated genes with more than 1.35 million distinct probes.

Microarray Gene Profile Data Analysis

Samples were processed and hybridized to the Affymetrix Human Gene 2.1 ST Array at the UNC core facility following the manufacturer's recommended protocols and reagents (Affymetrix). Data analysis was completed using GeneSpring software v.12.6 (Agilent Technologies). Two-way analysis of variance (ANOVA) statistical analysis was applied to determine differentially expressed genes among the various parameters (implant surface type and time points). Further analysis included pairwise comparisons of each implant surface independently at the different time points. Benjamini-Hochberg false discovery rate was used for multiple testing corrections. A P value of .05 was used as the threshold for statistical significance. To focus on the major gene expression changes, genes with more than twofold-changed expression compared with the control were analyzed.

Ingenuity Pathway Analysis

The dataset containing differentially expressed genes was uploaded into the Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, http://www.ingenuity.com) and was associated with the biologic functions and canonical pathways in the Ingenuity Knowledge Base. The well-characterized metabolic and cell signaling pathways have been curated and hand-drawn by the scientific community. Fisher's exact test ($P < .05$) was used to assess the significance of the associations between genes in the dataset and biologic functions or canonical pathways. In addition, for canonical pathways, a ratio was computed between the number of molecules from the dataset and the total number of molecules in that pathway. Beyond this statistical comparison, tables were compiled to contain the 10 most highly expressed genes (not including immunoglobulin genes) for each time point with respect to mucosa formed against laser-ablated abutments.

Clinical Assessment of Peri-implant Mucosal Healing

One year following implant and abutment placement, peri-implant probing depths were measured at machined and laser-modified abutment-supported implant crowns by a single investigator (A.L.). Six location probing measures were obtained at restored abutments with a UNC15 probe using moderate pressure in unanesthetized participants. Median values at each location were calculated and statistically compared using a Mann-Whitney Rank Order Test.

RESULTS

Implant and Participant Demographic

All participants (mean age = 53.65 years; range = 22 to 71 years) received two implants without implant failure. There were 11 men and 9 women. Among these participants, all peri-implant mucosal biopsy specimens were collected. Of the 40 biopsy specimens harvested in the 20 participants, processing of three tissues failed to produce RNA of quality to permit genome analysis (Table 1).

Microarray Data

In total, 718 genes were differentially expressed (fold change ≥ 2.0) when comparing abutment surface type among the four biopsy time points. Among these > 2.0-fold changes, 598 were statistically significant ($P \leq .05$). When comparing gene expression from tissues at machined versus laser-modified abutments at each time point, a total of 501 genes were differentially expressed (fold change ≥ 2.0), and 459 of these were statistically significant ($P \leq .05$).

Gene expression differences (> 2.0-fold change) in the healing tissues at the laser-modified versus machined implant abutments were compared at 1, 2, 4, and 8 weeks as shown in Tables 2 to 5. The upregulation of immunoglobulin genes was prevalent during the healing period, representing a majority of > 2.0-fold change genes for tissues at machined and laser-modified abutments, respectively. While acknowledging the role of B-cell mediated immunity in the biology of the peri-implant mucosa,[3,6] these genes were further excluded in the current data presentation. Other differences in the levels of gene expression in tissues at laser-modified implants between 1 week and weeks 2, 4, and 8 (> 2-fold differences) are presented in Table 6. Two genes encoding proteins specifically expressed in the JE, odontogenic ameloblast-associated protein (ODAM) and follicular dendritic cell secreted protein (FDCSP) were upregulated throughout the 8-week healing period.

Ingenuity Pathway Analysis

All peri-implant mucosa samples obtained adjacent to laser-ablated healing abutments (at time periods 1, 2, 4, and 8 weeks) were compared with machined 1-week samples, and canonical pathway analyses were created for all time points. Five top canonical pathways

Table 1 Participant Demographics and Implant Dimensions

				Machined abutment		Laser abutment	
Patient ID	Sex	Age (y)	Biopsy interval	Site[a]	Dimension (mm)	Site	Dimension (mm)
UNC-LL-01	M	48	8 wk	37	4.6 × 9	46	4.6 × 10.5
UNC-LL-02	M	36	8 wk	15	3.8 × 9	24[b]	4.6 × 10.5
UNC-LL-03	M	71	8 wk	21	4.6 × 10.5	23	4.6 × 10.5
UNC-LL-04	F	22	8 wk	35	4.6 × 9	45	3.8 × 10.5
UNC-LL-05	F	48	8 wk	15	4.6 × 10.5	25	3.8 × 10.5
UNC-LL-06	F	47	4 wk	34	3.8 × 9	46	4.6 × 9
UNC-LL-07	M	66	4 wk	47	4.6 × 10.5	24	3.8 × 10.5
UNC-LL-08	M	41	4 wk	16	4.6 × 9	26	4.6 × 10.5
UNC-LL-09	M	45	4 wk	24	4.6 × 10.5	16	4.6 × 9
UNC-LL-010	F	71	4 wk	36	4.6 × 10.5	45	4.6 × 10.5
UNC-LL-011	M	37	2 wk	25	3.8 × 9	35	4.6 × 10.5
UNC-LL-012	F	61	2 wk	46[b]	4.6 × 9	35	3.8 × 10.5
UNC-LL-013	M	67	2 wk	24	3.8 × 9	14	3.8 × 9
UNC-LL-014	F	47	2 wk	24	3.8 × 10.5	14	3.8 × 10.5
UNC-LL-015	M	57	2 wk	35	4.6 × 10.5	24	4.6 × 10.5
UNC-LL-016	M	56	1 wk	24	3.8 × 10.5	46	4.6 × 9
UNC-LL-017	F	65	1 wk	46	4.6 × 10.5	24	3.8 × 9
UNC-LL-018	M	64	1 wk	26	4.6 × 9	36	4.6 × 10.5
UNC-LL-019	F	65	1 wk	36	4.6 × 9	46	4.6 × 9
UNC-LL-020	F	59	1 wk	25[b]	3.8 × 9	15	3.8 × 9

[a]FDI tooth-numbering system.
[b]Tissue samples failed to produce RNA of quality to permit genome analysis.

Table 2 Upregulated Gene Expression at Laser-Modified Abutments at 1 Week

Transcript cluster ID	Gene symbol	Gene description	Fold change: laser-modified vs machined
167690976	–	–	2.37
16676682	IL24	–	2.27
16698764	–	–	2.10
16743721	MMP1	Matrixmetallopeptidase1 (interstitial collagenase)	2.05

Table 3 Upregulated Gene Expression at Laser-Modified Abutments at 2 Weeks

Transcript cluster ID	Gene symbol	Gene description	Fold change: laser-modified vs machined
16693308	FLG	Filaggrin	2.52
16693335	LCE3D	Late cornified envelope 3D	2.49
16693379	SPRRR2G	Small proline rich protein 2G	2.30
16844477	KRT10	Kerain 10	2.30
16671082	LCE1A	Late cornified envelope 1A	2.27
16693295	RPTN	Repetin	2.25
16693339	LCE3A	Late cornified envelope 3D	2.18
16671061	KPRP	Keratinocyte proline rich protein	2.16
16693331	LCE3E	Late cornified envelope 3E	2.13
16671041	LCE2C	Late cornified envelope 2C	2.12
16919538	WFDC12	WAP four-disulfide core domain 12	2.12

Table 4 Upregulated Gene Expression at Laser-Modified Abutments at 4 Weeks

Transcript cluster ID	Gene symbol	Gene description	Fold change: laser-modified vs machined
16765056	KRT76	Keratin 76	2.19
16671065	LCE1F	Late cornified envelope 1F	2.11
16844477	KRT10	Keratin 10	2.08

Table 5 Upregulated Gene Expression at Laser-Modified Abutments at 8 Weeks

Transcript cluster ID	Gene symbol	Gene description	Fold change: laser-modified vs machined
16844419	KRT24	Keratin 24	2.97
16814121	HBA2/HBA1	Hemoglobin alpha 2 / hemoglobin, alpha 1	2.57
17117579	–	–	2.47
16872611	CEACAM7	Carcinoembryonic antigen related cell adhesion molecule 7	2.33
16976821	PPBP	Pro-platelet basic protein (chemokine ligand 7)	2.19
16765116	KRT78	Keratin 78	2.12
16840799	ALOX12B	Arachidonate 12 / lipoxygenase, 12R type	2.10
16884602	IL36A	Interleukin36, alpha	2.09
16874693	KLK6	Kallikrein-related peptidase 6	2.07
16863419	IGFL1	IGF-like family member 1	2.04
16874751	KLK12	Kallikrein-related peptidase 12	2.02

were identified: *(1)* Granulocyte adhesion and diapedesis and *(2)* agranulocyte adhesion and diapedesis included 28 molecules, mostly cytokines related to cell adhesion and migration; *(3)* atherosclerosis signaling included 23 molecules, cytokines and transmembrane receptors related mainly to tissue and bone remodeling; *(4)* hepatic fibrosis/hepatic stellate cell activation included 19 molecules, mostly matrix metallopeptidases, which regulate different types of collagen and have cell migration, cell adhesion, and remodeling as important roles; *(5)* B Cell development included 10 molecules, mostly immunoglobulin transmembrane receptors (Table 7). IPA also identified five major signaling networks involving: *(1)* organismal injury and abnormalities (score: 36); *(2)* cell mediated immune response, cellular movement, hematologic system development and function (score: 30); *(3)* developmental disorder, hereditary disorder, skeletal and muscular disorders (score: 30); *(4)* connective tissue development and function, embryonic development, organ development (score: 28); *(5)* cell morphology, humoral immune response, dermatologic diseases and conditions (score: 25).

Clinical Assessments

During the 1-year evaluation period, no implant failure was recorded. All implants remained stable, and marginal bone level changes greater than 2 mm were not observed. Measurement of probing pocket depths trended to be shallower at the laser-modified versus machined implants with lingual probing pocket depths being statistically shallower ($P = .37$) (Table 8).

DISCUSSION

This study of peri-implant mucosa gene expression of differentially expressed genes in tissues forming at dental implant abutments with machined and laser-modified (Laser-Lok, BioHorizons) surfaces utilized an interim period biopsy technique to remove forming tissues at 1, 2, 4, and 8 weeks after implant and abutment placement. Using this approach in a split-mouth study, gene expression changes were successfully compared between tissues at abutments with and without laser modifications. Several other molecular assessments of tissue integration at implants exist. Ivanovski et al,[16] Thalji et al,[17] and Bryington et al[18] have utilized a genome-wide approach investigating the osseous responses to implanted dental implants in terms of surface micro- and nano-topography. Whole genome profiling allows for rapid and high-throughput quantification of thousands of genes simultaneously.[19]

The intent of this study was to define topography-dependent changes in the molecular progression of wound healing at dental implant abutments. A

Table 6 Change in Gene Expression (> 2-fold Change) from 1 Week to 2, 4, and 8 Weeks in Tissues at Laser-Modified Abutments

Transcript cluster ID	Gene symbol	Gene description
1 to 2 week change (top 10 upregulated genes)		
16917449	ODAM	Odontogenic, ameloblast associated
16743764	MMP13	Matrixmetallopeptidase 13 (collagenase3)
16967465	FDCSP	Follicular dendritic cell secreted protein
16743751	MMP12	Matrixmetallopeptidase 12 (macrophage elastase)
16967915	C4ORF26	Chromosome 4 open reading frame 26
16977045	CXCL9	Chemokine (C-X-C motif) ligand 9
16977052	CXCL10	Chemokine (C-X-C motif) ligand 10
16722562	SSA1	Serum amyloid A1
1683324	CCL2	Chemokine (C-C motif) ligand 2
16977058	CXCL11	Chemokine (C-X-C motif) ligand 11
1 to 4 week change (top 10 upregulated genes)		
16882707	LOC100294406	–
16967449	ODAM	Odontogenic, ameloblast associated
16927785	GGTLC2	Gamma-glutamyl transferase light chain 2
16968098	CXCL13	Chemokine (C-X-C motif) ligand 13
16967465	FDCSP	Follicular dendritic cell secreted protein
16743764	MMP13	Matrixmetallopeptidase 13 (collagenase3)
16967915	C4ORF26	Chromosome 4 open reading frame 26
17093536	CCL19	Chemokine (C-C motif) ligand 19
16977045	CXCL9	Chemokine (C-X-C motif) ligand 9
17064251	RARRES2	Retinoic acid receptor responder (tazarotene induced) 2
1 to 8 week change (top 10 upregulated genes)		
16967449	ODAM	Odontogenic, ameloblast associated
16967465	FDCSP	Follicular dendritic cell secreted protein
16900120	–	–
16722562	SSA1	Serum amyloid A1
16743751	MMP12	Matrixmetallopeptidase 12 (macrophage elastase)
16743764	MMP13	Matrixmetallopeptidase 13 (collagenase3)
16927785	GGTLC2	Gamma-glutamyl transferase light chain 2
16977045	CXCL9	Chemokine (C-X-C motif) ligand 9
16967915	C4ORF26	Chromosome 4 open reading frame 26
16977052	CXCL10	Chemokine (C-X-C motif) ligand 11

Table 7 List of Top 5 Canonical Pathways Identified in Healing Tissues Adjacent to Laser-Modified Abutments

Canonical pathway	No. of included molecules	*P* value	Ratio
Granulocyte adhesion and diapedesis	28	1.07×10^{-16}	28/182 (0.154)
Agranulocyte adhesion and diapedesis	28	5.52×10^{-16}	28/192 (0.146)
Atherosclerosis signaling	23	1.72×10^{-15}	23/139 (0.165)
Hepatic fibrosis/hepatic stellate cell activation	19	1.8×10^{-10}	19/155 (0.123)
B cell development	10	8.79×10^{-10}	10/36 (0.278)

Table 8 Peri-implant Sulcus Probing Depths at 12 Months Following Implant and Abutment Placement

	Facial	Mesiobuccal	Mesiolingual	Lingual	Distolingual	Mesiolingual
Laser-modified	1.7 (0.7) mm	2.4 (1.2) mm	2.5 (1.5) mm	1.6 (0.7) mm*	2.4 (1.4) mm	2.3 (1.1) mm
Machined	1.9 (1.1) mm	2.7 (0.8) mm	2.8 (0.9) mm	2.4 (0.97) mm	2.6 (0.83) mm	2.7 (0.95) mm

*P = .037; Wilcoxon Rank Order Test.

time-dependent series of cellular events occurred in a reproducible manner and reflected the physiology of the distinct epithelial, vascular, immunologic, and fibroblastic components of the cells that comprise the formed biologic width around dental implants. A prominent role of B-cells is assumed, based upon the large number of upregulated immunoglobulin genes present in tissues at all times at both implant surfaces. This is consistent with the population of cells defined within the peri-implant connective tissue.[20]

IPA analysis demonstrated that a wound healing process that involves cellular recognition of the alloplastic surface is associated with topography-dependent changes in the formed tissues. Several proteins expressed selectively by the oral keratinized epithelium are represented among the genes with expression elevated greater than twofold during healing at both abutment types. These included the small proline-rich proteins (SPRP) with defined protective functions in skin and mucosa, and different members of the late cornified envelope (LCE) genes. These keratinocyte-associated proteins underscore the role of the stratum corneum in barrier function. Together with transglutaminases, small proline-rich proteins, and loricrin, they are cross-linked into a protective barrier of keratinized mucosa. In wound healing, their expression is often upregulated.[21] The gingival epithelium and the peri-implant mucosal epithelium are both composed of parakeratinized stratified epithelia that cover the external surface of the gingiva. Clearly, the pattern of gene regulation included consistent and elevated keratinocyte protein gene expression.

The increased epithelial gene expression that occurred during the 2- to 8-week period reflects the temporal process of epithelial attachment formation revealed in human histologic studies.[1,9] In tissues adjacent to laser-modified titanium abutments, elevated levels of several structural and functional protein-encoding mRNAs specific to the cornfield epithelium were observed (Tables 2 to 5). At 2 weeks, upregulation of epithelium-specific proteins involved in protection implies that early healing at implant abutments involves epithelial protective function. At 8 weeks, a specific cell adhesion molecule CEACAM7 was upregulated in the group of laser-modified tissues. The CEACAM protein family mediates intercellular attachment of the JE. CEACAM7 was most highly induced protein when comparing laser-ablated to machined surfaces at 8 weeks, suggesting the favorable development or maturation of the JE at the laser-modified surface at this time point.

The JE plays a major role in the tissue-abutment interface function.[6,22] Importantly, this study revealed the upregulated expression of genes involved in JE formation. ODAM is uniquely expressed in the JE. It may influence the cellular events during the apical extension of the gingival wound edge to reestablish a functional JE.[23] Here, ODAM is highly expressed throughout the healing phase. Another protein, FDCSP, is also highly expressed during this 8-week healing period. It is a small secretory protein related to the salivary protein, statherin, and is specifically expressed by cells of the JE. Dabija-Wolter et al[24] revealed that both ODAM and FDCSP are JE-specific markers. Here, both are elevated in expression within tissues forming at the implant abutments. The authors of the present study conclude that JE formation is a prominent part of peri-implant mucosal wound healing.

Multiple chemokine mRNAs were also upregulated during the 8-week healing period. This underscores the importance of cell-to-cell interactions in inflammatory regulation in wound healing. Chemokines, mediators of mesenchymal/epithelial cell interactions, were specifically expressed during wound healing. For example, there are significant differences between skin and oral epithelium (tongue) healing. CCL5, CCL12, and CXCL10 only appeared in tongue wounds, while CCL3, CCL20, CXCL3, CXCL7, and CXCL13 only appeared in skin wounds.[25] Consistent with these findings, here CXCL10 and CCL12 are expressed in oral mucosa healing at abutments. Interestingly, Chen et al[25] found no expression of MMP1 and MMP13 during oral wound healing, and this differs from the greater than twofold increase observed here during peri-implant mucosal healing. Healing at titanium abutments may involve altered patterns of gene expression that includes upregulation of MMP1. MMP1 is expressed by epithelial cells, and its presently elevated expression underscored the importance of surface-mediated modulation of epithelial function during establishment of the implant abutment/mucosal interface.

Few previous reports reference regulated gene expression that occurs within forming peri-implant mucosa. An et al[26] examined the expression of select

genes believed to be specific to the JE. The expression of CK14, vinculin, integrin a6b4, transforming growth factor (TGF)-β, TGF-α1, and TGF-β 3 differed as a function of surface hydrophobicity (sandblasted, large-grit acid-etched [SLA] vs modSLA surfaces). Genome-wide association study (GWAS) was used to compare tissue healing over 12 weeks at abutments following one- versus two-stage surgery performed in minipigs.[27] The authors concluded that flapless surgery was associated with upregulation of detoxification and re-epithelialization genes, and myofibroblast genes were upregulated in the flapped surgery tissues. Unfortunately, little gene expression data were reported.

A conspicuous absence of significant upregulated gene expression involving fibroblastic adhesion was noted, and a prominent role of epithelium in the initial process of mucosal integration is suggested. Alternatively, the circumferential biopsy method may preferentially represent epithelial components at the expense of connective tissue. While this potentially limits the scope of the investigation, the observations made regarding epithelium-specific gene regulation are relevant to the process of implant abutment/mucosa integration.

Peri-implant mucosal collagen fibrillogenesis may be influenced by implant-abutment topography. Surface-specific alterations in collagen fibril formation may underscore observed differences in collagen organization in the connective tissue within the mucosa formed at laser-modified versus machined dental implant abutments.[14] In a study conducted in foxhounds, after 3 months of healing, histologic evaluation demonstrated that compared with machined abutments, the mucosa formed at laser-modified abutments contained a dense interlacing complex of connective tissue fibers oriented relatively perpendicular to the abutment surface.[15] Similar results were also replicated in later human case studies.[28,29] Given the importance recently placed on collagen fibril orientation and organization following peri-implant mucosa healing in canines and humans,[15,28] there is value in considering collagen fibrillogenesis. Here, mRNAs encoding proteins that regulate collagen fibril formation were elevated more than twofold greater in tissues from laser-modified than machined abutments (Table 9). mRNAs with higher expression in tissues at laser-ablated versus machined abutments imply a period of intensive collagen biosynthesis and collagen fibril organization and included: *(1)* extracellular matrix collagens or collagen-associated proteins (eg, SIBLINGs and collagens); *(2)* proteins involved in the process of fibrillogenesis (asporin, osteoglycin, osteomodulin, MMP13); or *(3)* proteins that regulate cellular collagenesis (eg, ADAM28, ADAM6, and FGF7). All may contribute to surface-dependent modulation or modification of the process of peri-implant abutment/mucosa integration. This reflects what is known from histologic demonstration of connective tissue organization in the forming peri-implant mucosa that occurs at 4 to 6 weeks.[30]

Table 9 Upregulated Expression of mRNAs Encoding Proteins Associated with Collagen Biosynthesis

Laser-modified/machined		
2 wk; > 2.0 FC	**4 wk; > 2.0 FC**	**8 wk; > 2.0 FC**
EMC2	MMP13	ADAM28
SPARC	Col1A	ADAM6
ADAM28	Osteoglycin	
	ADAM6	
	Asporin	
	Osteomodulin	
	Col14A	
	Col1A2	
	IBSP	
	ColIA1	
	FGF7	

Several clinical observations were made in this human clinical study. Subjectively, the harvesting of tissues was complicated by the difficult dissection of tissues from the laser-modified abutments but not machined abutments at the 8-week time point. This could reflect the intimate relationship of fibrous tissues with the laser-modified surfaces.[15] This clinical relationship, while beyond the intended scope of this study, underscores that topographically distinct abutments altered the initial process of peri-implant abutment/mucosal wound healing. Objective differences were observed at the level of pocket probing depths measured at 1 year following implant and abutment placement. The shallower probing depths measured at six points around laser-modified abutments versus machined abutments is consistent with altered dental implant abutment/mucosal integration. Because this study utilized implants without laser-modified surfaces, the modest differences in probing pocket depths are presently attributed to the different abutment surfaces used in this investigation.

CONCLUSIONS

This clinical comparison of mucosal wound healing at laser-modified versus machined abutments demonstrated that the topographic nature of the implant abutment influences mucosal wound healing. Generally, the molecular process of implant abutment/mucosa integration involves a prominent B-cell response, marked upregulation of keratinized

and JE gene expression, and collagen fibril formation. Isotropic laser-modification of a 0.7-mm abutment surface influences JE formation and collagen biosynthesis. A significant reduction in probing pocket depths at laser-modified implant abutments compared with machined abutments 1 year following implant and abutment placement was observed. Surface topography represents a viable approach to modifying peri-implant mucosal tissue responses during wound healing.

ACKNOWLEDGMENTS

This study was funded by BioHorizons, Inc. The authors thank Ms Gidget Jenkins, clinical study coordinator, and Ruiwei Luang, DDS, PhD for her assistance in mRNA sample preparation. The authors reported no conflicts of interest related to this study.

REFERENCES

1. Tomasi C, Tessarolo F, Caola I, et al. Early healing of peri-implant mucosa in man. J Clin Periodontol 2016;43:816–824.
2. Gargiulo AW, Wentz FM, Orban B. Dimensions and relations of the dentogingival junction in humans. J Periodontol 1961;32:261–267.
3. Welander M, Abrahamsson I, Berglundh T. The mucosal barrier at implant abutments of different materials. Clin Oral Implants Res 2008;19:635–641.
4. Ericsson I, Persson LG, Berglundh T, Marinello CP, Lindhe J, Klinge B. Different types of inflammatory reactions in peri-implant soft tissues. J Clin Periodontol 1995;22:255–261.
5. Broggini N, McManus LM, Hermann JS, et al. Peri-implant inflammation defined by the implant-abutment interface. J Dent Res 2006;85:473–478.
6. Zitzmann NU, Berglundh T, Marinello CP, Lindhe J. Experimental peri-implant mucositis in man. J Clin Periodontol 2001;28:517–523.
7. Berglundh T, Lindhe J. Dimension of the periimplant mucosa. Biological width revisited. J Clin Periodontol 1996;23:971–973.
8. Klinge B, Meyle J; Working Group 2. Soft-tissue integration of implants: Consensus report of Working Group 2. Clin Oral Implants Res 2006;17(suppl):s93–s96.
9. Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljenberg B, Thomsen P. The soft tissue barrier at implants and teeth. Clin Oral Implants Res 1991;2:81–90.
10. Atieh MA, Ibrahim HM, Atieh AH. Platform switching for marginal bone preservation around dental implants: A systematic review and meta-analysis. J Periodontol 2010;81:1350–1366.
11. van Brakel R, Meijer GJ, Verhoeven JW, Jansen J, de Putter C, Cune MS. Soft tissue response to zirconia and titanium implant abutments: An in vivo within-subject comparison. J Clin Periodontol 2012;39:995–1001.
12. Barwacz CA, Brogden KA, Stanford CM, Dawson DV, Recker EN, Blanchette D. Comparison of pro-inflammatory cytokines and bone metabolism mediators around titanium and zirconia dental implant abutments following a minimum of 6 months of clinical function. Clin Oral Implants Res 2015;26:e35–e41.
13. Delgado-Ruiz RA, Calvo-Guirado JL, Abboud M, et al. Connective tissue characteristics around healing abutments of different geometries: New methodological technique under circularly polarized light. Clin Implant Dent Relat Res 2015;17:667–680.
14. Nevins M, Nevins ML, Camelo M, Boyesen JL, Kim DM. Human histologic evidence of a connective tissue attachment to a dental implant. Int J Periodontics Restorative Dent 2008;28:111–121.
15. Nevins M, Kim DM, Jun SH, Guze K, Schupbach P, Nevins ML. Histologic evidence of a connective tissue attachment to laser microgrooved abutments: A canine study. Int J Periodontics Restorative Dent 2010;30:245–255.
16. Ivanovski S, Hamlet S, Salvi GE, et al. Transcriptional profiling of osseointegration in humans. Clin Oral Implants Res 2011;22:373–381.
17. Thalji GN, Nares S, Cooper LF. Early molecular assessment of osseointegration in humans. Clin Oral Implants Res 2014;25:1273–1285.
18. Bryington M, Mendonça G, Nares S, Cooper LF. Osteoblastic and cytokine gene expression of implant-adherent cells in humans. Clin Oral Implants Res 2014;25:52–58.
19. Stears RL, Martinsky T, Schena M. Trends in microarray analysis. Nat Med 2003;9:140–145.
20. Gualini F, Berglundh T. Immunohistochemical characteristics of inflammatory lesions at implants. J Clin Periodontol 2003;30:14–18.
21. de Koning HD, van den Bogaard EH, Bergboer JG, et al. Expression profile of cornified envelope structural proteins and keratinocyte differentiation-regulating proteins during skin barrier repair. Br J Dermatol 2012;166:1245–1254.
22. Bosshardt DD, Lang NP. The junctional epithelium: From health to disease. J Dent Res 2005;84:9–20.
23. Nishio C, Wazen R, Kuroda S, Moffatt P, Nanci A. Expression pattern of odontogenic ameloblast-associated and amelotin during formation and regeneration of the junctional epithelium. Eur Cell Mater 2010;20:393–402.
24. Dabija-Wolter G, Bakken V, Cimpan MR, Johannessen AC, Costea DE. In vitro reconstruction of human junctional and sulcular epithelium. J Oral Pathol Med 2013;42:396–404.
25. Chen L, Arbieva ZH, Guo S, Marucha PT, Mustoe TA, DiPietro LA. Positional differences in the wound transcriptome of skin and oral mucosa. BMC Genomics 2010;11:471.
26. An N, Rausch-fan X, Wieland M, Matejka M, Andrukhov O, Schedle A. Initial attachment, subsequent cell proliferation/viability and gene expression of epithelial cells related to attachment and wound healing in response to different titanium surfaces. Dent Mater 2012;28:1207–1214.
27. Mueller CK, Thorwarth M, Chen J, Schultze-Mosgau S. A laboratory study comparing the effect of ridge exposure using tissue punch versus mucoperiosteal flap on the formation of the implant-epithelial junction. Oral Surg Oral Med Oral Pathol Oral Radiol 2012;114(suppl):s41–s45.
28. Nevins M, Camelo M, Nevins ML, Schupbach P, Kim DM. Connective tissue attachment to laser-microgrooved abutments: A human histologic case report. Int J Periodontics Restorative Dent 2012;32:384–392.
29. Geurs NC, Vassilopoulos PJ, Reddy MS. Histologic evidence of connective tissue integration on laser microgrooved abutments in humans. Clin Adv Periodontics 2011;1:29–33.
30. Berglundh T, Abrahamsson I, Welander M, Lang NP, Lindhe J. Morphogenesis of the peri-implant mucosa: An experimental study in dogs. Clin Oral Implants Res 2007;18:1–8.