Comparison of Pro-Inflammatory Cytokines and Bone Metabolism Mediators Around Laser-Lok and Machined Transmucosal Abutments: A Pilot, Randomized Clinical Trial

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Background

Surface microgeometry, and its role in modulation of cellular behavior are increasingly gaining attention for use in industrial, military, and biomedical applications. Such technology has recently been incorporated into implant dentistry. Biohorizons® is the first implant company to incorporate surface microgeometry in both an implant fixture and prosthetic abutment. It has been demonstrated both histologically[1] and clinically[2-4] that 8μm laser-ablated Laser-Lok microchannels spanning 1.8-2.1mm at the coronal aspect of the implant fixture, and 0.7mm at the apical-most portion of the abutment, lead to enhanced bone and soft-tissue responses compared with traditional machined surfaces.

Studies have attributed this superior bone and peri-implant mucosa healing response to the biomimetic orientation of the microgrooves, which leads to peri-implant connective tissue attachment in an oblique, rather than concentric/parallel orientation to the implant/abutment interface. [5-7] It may be hypothesized that such a change in fiber orientation achieves two ends: 1) The apposition of a robust, obliquely-oriented connective tissue compartment provides an anatomical barrier to apical junctional epithelium migration, thus potentially minimizing marginal bone resorption at early healing stages; 2) An oblique orientation of connective tissue may prevent or reduce the penetrance of microbial agents, and subsequent deleterious effects that pro-inflammatory mediators have in the peri-implant sulcus microenvironment in the long-term.

Although the aforementioned benefits are plausible on the basis of available histological, clinical, and radiographic studies in dogs and humans, [8] further translational clinical research is needed to understand the impact that this technology has on clinical and biological outcomes. Specifically, there have been no human clinical trials evaluating the impact that Laser-Lok abutments have in the regulation or expression of pro-inflammatory cytokines and bone metabolism mediators in the adjacent peri-implant crevicular fluid (PICF) at different stages of implant therapy. Addressing this gap of knowledge with a prospective, randomized clinical trial would be beneficial to gain knowledge regarding the intimate molecular mechanisms by which Laser-Lok abutments may lead to superior clinical outcomes.

Rationale for PICF analysis of Laser-Lok abutments

Requisite to the long-term stability of an implant-supported restoration is the use of prosthetic materials possessing optimal biomechanical and biocompatible characteristics. [9-12] An implant's transmucosal abutment highlights these requirements, as it must span three distinct interfaces (implant-abutment interface, transmucosal connective and epithelial tissues, and the free gingival margin). Therefore, the ideal transmucosal abutment should, as a result of its material properties, contribute to long-

term homeostasis of the peri-implant mucosal microenvironment. This can be promoted via the suppression or downregulation of pro-inflammatory mediators (e.g., cytokines, chemokines, and bone markers) that may be released in a paracrine fashion by cells of the peri-implant mucosa in response to a given abutment biomaterial. To date, analyses via PICF sampling techniques, such as paramagnetic bead [13] and filter paper strip collection [14] have been instrumental in determining the role that biomarkers play in the pathogenesis and progression of peri-implant diseases. Investigators have evaluated various conditional parameters as they relate to PICF dynamics, including comparison of samples obtained from healthy dental implants and natural teeth[15,16], effects of de novo plaque-induced peri-implant mucositis,[17,18] established peri-implant mucositis,[19] peri-implantitis,[20-22] influence of genotypic characteristics,[23,24] localization of the prosthetic microgap,[25] and effects of smoking.[26]

Consistently, these studies have demonstrated that the expression of biomarkers indicative of alterations in tissue homeostasis (e.g., pro-inflammatory mediators or bone turnover markers) can be quantified under conditions associated with localized acute or chronic inflammation. To this end, some of the authors of this proposal have recently conducted a PICF-based clinical trial at The University of Iowa, College of Dentistry comparing pro-inflammatory cytokines and bone-metabolism mediators around titanium and zirconia definitive abutments that have been in clinical function for a minimum of six months.[27]

Study Design Rationale

A pilot, prospective, randomized clinical trial is proposed **(Figure 1).** It is primarily aimed at assessing the expression of pro-inflammatory cytokine **(Figure 2A)** and bone metabolism mediators **(Figure 2B)** adjacent to Laser-Lok microgrooved (LL) or machined (M) transmucosal healing abutments. The pilot study would seek recruitment of 12 subjects. The pilot study is designed to investigate two principle aims driven by current gaps in knowledge:

Gap of Knowledge #1: Do obliquely oriented connective tissue fibers reduce or eliminate the negative pro-inflammatory sequelae (i.e., crestal bone remodeling and peri-implant mucosa inflammation) commonly found at the implant-abutment junction during healing? How do these values compare with natural tooth controls in the same patient?

Aim 1. Characterize pro-inflammatory and bone-metabolism mediator profiles adjacent to Laser-Lok microgrooved (LL) and machined (M) healing abutments as well as natural teeth after a period of healing (8 weeks) via paper strip-based PICF sampling.

Gap of Knowledge #2: Does the quantity of PICF volume differ around functionally-oriented fibers in the mucosa around Laser-Lok microgrooved (LL) abutments as

compared with machined (M) healing abutments? How do these volumes compare with natural teeth, which have been shown to have less volume as compared with implants?

Aim 2. Utilize the Periotron 8000 (Oraflow, Inc., Smithtown, NY, USA) to quantify PICF sample volume at 8 weeks post-implant placement to evaluate if functionally-oriented mucosa (analogous to natural teeth) around Laser-Lok microgrooved abutments reduce the volumetric production of PICF, as compared with standard machined abutments, relative to a natural tooth control.

Materials and Methods

Study Population:

The investigators propose to recruit 12 subjects requiring replacement of a single, tooth-bound molar or premolar in the maxilla or mandible with an implant-supported restoration.

Inclusion Criteria

- 18 years of age or greater
- Subjects requiring the replacement of either a tooth-bound molar or premolar in either arch that do not require simultaneous implant site development (i.e., bone and/or soft-tissue grafting)
- Teeth adjacent (mesial and distal) to study site must consist of two stable, natural teeth without signs of periodontal attachment loss up to 2.0mm
- An opposing dentition with teeth, implants, or fixed prosthesis
- Subjects must be willing to forgo use of a provisional appliance (e.g., removable interim partial denture (or) "flipper", essix appliance, etc.) during the active portion (from implant placement to 8 week +IP visit) of the study protocol.
- Subjects must be willing to follow instructions related to the study procedures
- Subjects must have read, understood, and signed the informed consent document

Exclusion Criteria

- Insufficient interocclusal space for implant placement and/or restoration at study site
- Insufficient lateral ridge volume for implant placement in a prosthetically-driven location
- More than 2.0 mm of vertical bone loss at study site as measured from the interproximal crestal bone on the adjacent teeth
- Untreated rampant caries
- Tobacco use free for ≤ 6 months
- Liver or kidney dysfunction/failure

- Active severe infectious diseases that may affect normal healing and/or bone metabolism
- Uncontrolled diabetes determined as HbA1c value > 7%
- Current alcohol or drug abuse
- Need for systemic medications (e.g., corticosteroids) that may influence post-operative healing and/or osseointegration
- History of relevant head/neck cancer and/or radiation of the head/neck within the last 24 months
- Subjects who currently use IV bisphosphonates or have a history of IV bisphosphonate use
- Subjects with metabolic bone diseases such as severe osteoporosis or Paget's disease of bone
- Known pregnancy or nursing mothers
- Unwilling to forgo use of a provisional appliance (e.g., removable interim partial denture (or) "flipper", essix appliance, etc.) during the active portion (from implant placement to 8 week +IP visit) of the study protocol.
- Unable or unwilling to return for follow-up visits for a period of 1 year
- Unlikely to be able to comply with study procedures according to investigators judgement

Surgical Procedures

A total of 12 implants (one per subject) will be placed in premolar and molar sites under local anesthesia following a conventional approach, in a non-submerged fashion. Implants should demonstrate primary stability at the time of placement, defined as a minimum insertion torque of 32 Ncm^2. If primary stabilization of an implant cannot be achieved, the standard of care is to attempt placement of a larger (wider, longer, or both) implant in order to achieve primary stability or, if not possible, to graft the area, abort the implant placement at that time and allow further healing for an additional period of time (at least 3 months) prior to attempt implant placement again. Peri-implant mucosal tissues will be stabilized with a double-sling suturing technique. Subjects will receive verbal and written post-operative instructions. Patients will be asked to avoid direct contact with the surgical site for the first week. Regular oral hygiene measures in the rest of the oral cavity will be encouraged. Specific oral hygiene instructions may also include the use of 0.12% chlorhexidine mouth rinses to reduce risk of oral infection in populations at higher risk, as determined by the investigators. Patients will be prescribed oral antibiotics, Amoxicillin 500 mg every 8 hours for 7 days (if allergic: Azithromycin 250mg 2 tablets first day then 1 tablet/day for the remaining 4 days) and an anti-inflammatory and pain reliever drug: Ibuprofen 600 mg, every 6 hours for 3 to 5

days. Sutures will be removed at approximately 1 week postoperatively, and at that time oral hygiene instructions will be reinforced.

Peri-implant crevicular fluid (PICF) and Gingival Crevicular Fluid (GCF) sampling

Each subject's implant site and corresponding natural tooth will be isolated with cotton rolls, and light air will be applied over the site to eliminate ambient salivary contamination of the PICF/GCF sample. Sampling will occur for 30 seconds at two distinct sites (buccal and lingual) of the healing abutment and natural tooth by one clinician under loupe magnification. The paper strips (PerioPaper Strips, Oraflow Inc., Smithtown, NY, USA) will be inserted with cotton forceps into the gingival crevice until mild resistance is felt. Upon termination of sampling, PICF and GCF volumes will be immediately quantified for each strip using the Periotron 8000 Instrument (Oraflow, Inc., Smithtown, NY, USA), which is calibrated using known volumes of buffer. In cases of visible contamination with blood, the strip will be discarded. Each strip will then be placed in an empty sterile microcentrifuge tube (Seal Rite, USA Scientific Inc., Ocala, FL, USA) and frozen at -80°C.

Cytokine and bone metabolism mediator quantities (pg/30 s) will be determined using a commercial 6-multiplexed fluorescent bead-based immunoassay (Kit HBNMAG-51K, Millipore, Billerica, MA) and the Luminex 100 IS Instrument (Luminex, Austin, TX, USA). This specific 6-multiplex kit is capable of detecting specific pro-inflammatory cytokines, chemokines, and bone metabolism mediators **(Figure 2B)**.

Two PICF samples per patient will be warmed from -80°C on ice. Each sample will be resuspended in 75 µl cold 0.01 M PBS, pH 7.2 and protease inhibitor (Complete Mini, protease inhibitor cocktail tablets, Roche Applied Science, IN, USA). Samples will be vortex mixed for 10 s and placed on a shaker for 20 min. at 4°C. The tubes will be centrifuged for 5 min. at 3,220 g (4,000 rpm) to pellet the strip, plaque, and cellular debris. The contents of each of the two samples per patient will then be removed and pooled into a single microcentrifuge tube (Seal Rite, USA Scientific Inc., Ocala, FL, USA).

For the assay, 25 µl aliquots of 0.01M PBS, pH 7.2 containing PICF samples will be incubated with anti-human multi-cytokine beads for 4°C for 18 h. Unbound antigen will be removed by filtration. Anti-human multi-cytokine biotin reporter will be added, and reactions will be incubated at room temperature for 1.5 h in the dark. Streptavidin-phycoerythrin will then be added, and the plates will be incubated at room temperature for an additional 30 min. Stop solution will be added, and the plates read in a plate reader (Luminex 100 IS, Luminex, Austin, TX, USA). Cytokine quantities in each sample will be extrapolated based on standards utilizing Beadview software (Millipore, Billerica, MA).

Radiographs

The authors propose to utilize standardized periapical radiography at specific key time points (IP, 8 weeks, and at final restoration delivery). Standardized periapical radiography would commence the day of implant placement by fabrication of a custom bite-registration positioning jig attached to a position indicating device (PID). Standardized radiographs would enable precise comparisons of crestal bone level changes throughout the course of the study. The rationale for the timepoints of 8 weeks and 12 weeks post-IP is to allow for 8 weeks of time to pass under a specific conditional parameter (i.e., LL vs. M abutment placement post-IP (Aim 1) and to verify removal of excess cement and proper seating of prosthetic components at restoration delivery.

Clinical Photodocumentation

At the conclusion of the active phase of the trial (8 weeks), patients will have an implant-level impression and receive a definitive Laser-Lok esthetic abutment and abutment-supported crown within 4 weeks. Clinical photographic documentation of abutments and restorations will be completed. A study plan events summary is represented in Figure 3.

Statistical Methods

The 95% confidence interval of the effect of Laser-Lok (LL) relative to machined (M) healing abutments on the pro-inflammatory cytokine and bone mediator variables will be computed. Since the cytokine and bone mediator variables have a log-normal distribution, the natural log (Ln) transformation of these variables will be used in the analysis, where the 95% CI for the mean difference in the Ln scale between LL and M will be calculated. For interpretation based on the original scale, the exponential function will be applied to the mean difference and the 95% confidence limits in the Ln scale. This estimate will provide the mean ratio (95% CI) of cytokine (or mediator) for LL relative to M. To interpret this 95% CI of the mean ratio of LL/M, an interval that is shifted more in the direction of less than 1 ratio suggests an effect with possible lower levels for LL relative to M. Otherwise, a 95% interval that is shifted more in the direction of greater than 1 ratio suggests an effect with possible greater levels for LL relative to M.

Note that n=6 per group will produce wide CI, based on the SD that we had computed from our previous studies, the (lower, upper) limit of the 95% CI of the mean ratio are as follows,
OPN: (mean ratio) x 0.78 , (mean ratio) x 1.27
PTH: (mean ratio) x 0.52 , (mean ratio) x 1.93
TNF-a: (mean ratio) x 0.44 , (mean ratio) x 2.25
IL1-b: (mean ratio) x 0.15 , (mean ratio) x 6.55

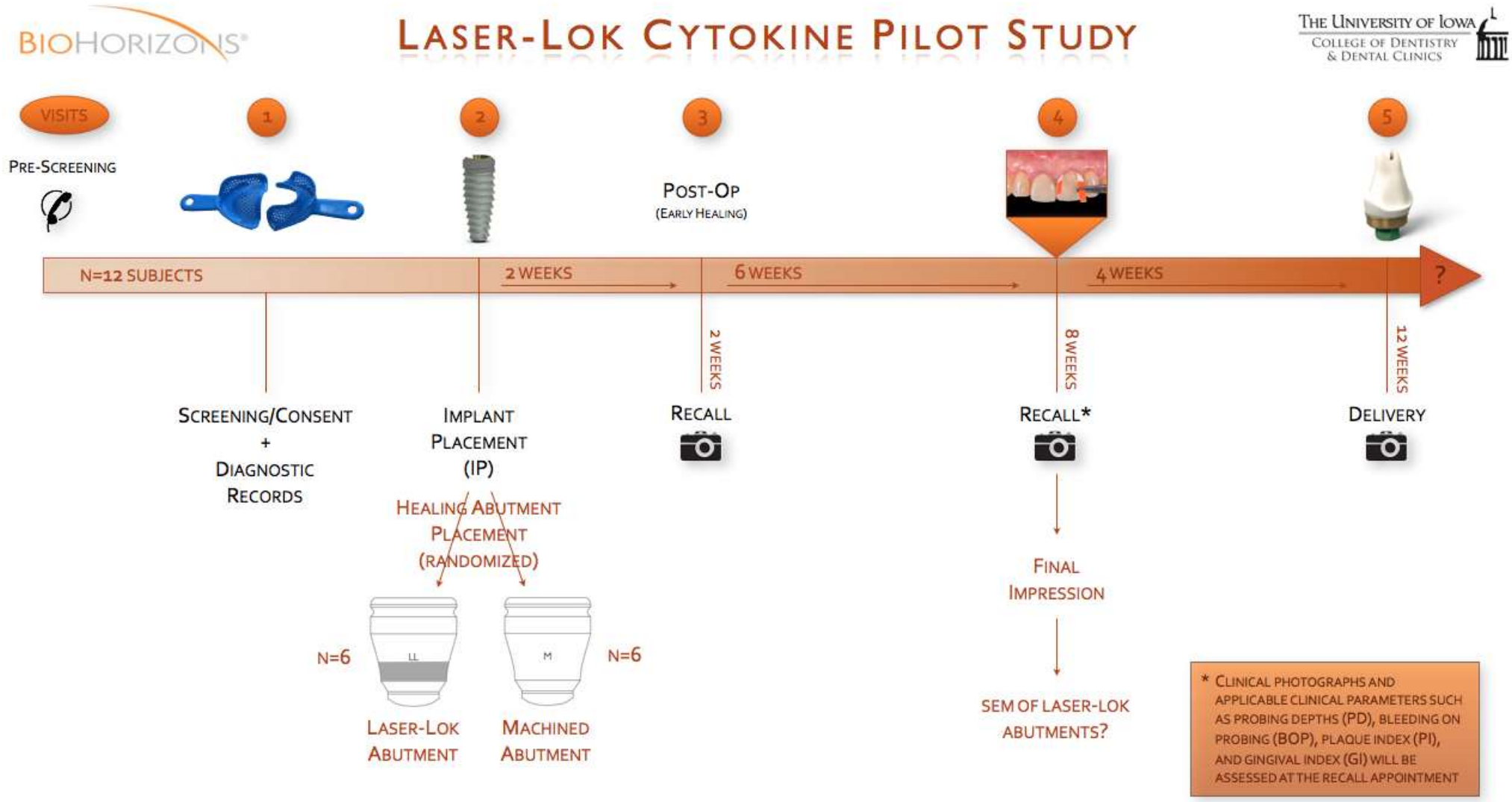

Figure 1: Proposed Study Sequence Overview

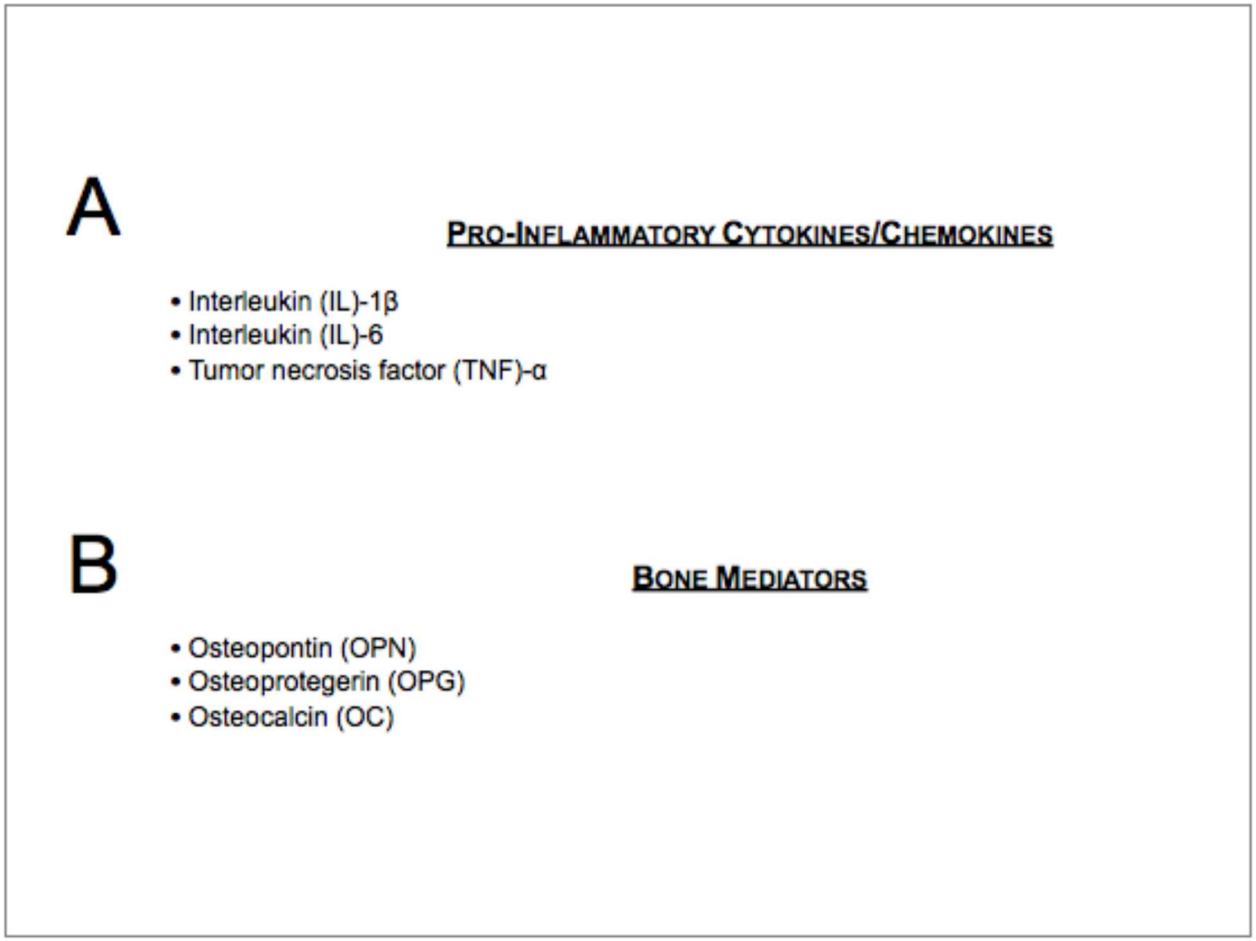

Figure 2: Proposed pro-inflammatory cytokines (A) and bone-mediators (B) to evaluate and compare in the peri-implant crevicular fluid adjacent to Laser-Lok and machined-surface healing abutments.

	Visit	1	2	3	4	5
	Visit Description	Screening	Implant Placement (IP)	Recall Visit (Early Healing)	Recall Visit (PICF Sampling)	Permanent Crown
	Visit Window		IP	IP + 2w	IP + 8w	IP + 12w
Selection process	Informed Consent	X				
	Medical/Dental history	X				
	Oral examination	X				
	Inclusion/exclusion criteria	X				
	Radiographic examination	X				
Study outcomes	Implant Stability		X	X	X	X
	Clinical Photographs		X	X	X	X
	Standardized Radiograph		X			X
	Peri-Implant Crevicular Fluid (PICF) Sampling				X	
	Plaque Index (PI), Gingival Index (GI), Periodontal Probing Depths (PPD), Bleeding on Probing (BOP)				X	
	Healing Abutment Randomization		X			
	Adverse Events/Adverse Device Effects		X	X	X	X

Figure 3: Proposed study plan of events by visit and study outcome measure.