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Treatment of peri-implant mucositis and supportive peri-implant therapy using an erythritol air- polishing device versus conventional mechanical instrumentation. A 6-month randomized controlled clinical trial.

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General Investigator Plan

The study will be conducted at The University of Michigan School of Dentistry, Department of Periodontics and Oral Medicine, Graduate Periodontics Clinic. Research subjects will be recruited from patients seeking and/or receiving dental care at the University of Michigan Graduate Periodontics department. Forty participants with dental implants diagnosed with peri-implant mucositis are expected to join the research study. Patient information will be protected according to the privacy regulations of the Federal Health Insurance Portability and Accountability Act of 1996 (HIPAA).

Study Protocol

I. Introduction/Background

Biological complications around dental implants are often present as inflammation of the peri-implant mucosa and alveolar bone occurs in response to the accumulation of bacterial biofilm

Peri-implant tissue shares similar oral environment with natural teeth. In fact, dental implants have similar bacteria colonized as natural dentition and smokers have increased levels of *Porphyromonas gingivalis (Pg)*, *Parvimonas micra*, and *Fusobacterium nucleatum* (Stokman et al. 2016). However, Limited evidence showed that microbiota in peri-implant diseases is related to *Pg*, *Treponema denticola*, and *Tannerella forsythia*. In contrast, some evidence supports the presence of *Prevotella intermedia* (Jervoe-Storm et al. 2015) and *Camplylobacter rectus* (Perez-Chaparro et al. 2016) in peri-implantitis.

Periodontal pathogens may play important role in the shift from healthy implant status to peri-implant mucositis, an early transitional state during the progression of peri-implantitis (Zheng et al. 2015). Early detection and resolution of inflammatory factors of peri-mucositis has become crucial to prevent to peri-implantitis, which involve irreversible destruction of peri-implant tissue. Effective peri-implant maintenance therapy is required to prevent complications and improve the long-term success of implants (Monje et al. 2016; Yeung et al. 2008).

Case definition of peri-implant mucositis requires presence of bleeding (BOP) and/or suppuration on gentle probing with or without increased probing depth (PD) compared to existing examinations (Berglundh et al. 2018a). An increase in probing depth may also occur due to swelling or reduction of probing resistance in peri-implant tissue (Berglundh et al. 2018b). Prevalence of peri-implant mucositis varied widely, ranging from 63.4% and 30.7% at patient and implant level with a mean of 43% (Atieh et al. 2013; Derks and Tomasi 2015).

In addition, bone loss beyond initial bone remodeling should not be detected in peri-implant mucositis. Peri-implant diseases have been associated with an increase in gram-negative bacteria which is similar to periodontal pathogenesis in natural teeth (Pontoriero et al. 1994). Hence, elimination of adherent biofilm around the dental implant is the primary objective in the treatment of peri-implant mucositis. Mechanical therapy with or without adjunctive use of antimicrobial or antiseptic agents is considered as the initial treatment of choice for management of peri-implant mucositis (Renvert and Polyzois 2015). Locally delivered antimicrobial, antiseptic rinse, antiseptic gel or irrigation have been used as adjunctive agents to mechanical debridement (Porras et al. 2002; Schenk et al. 1997). However,

these have failed to prove additional advantage over mechanical therapy alone in the treatment of peri-implant mucositis (Heitz-Mayfield et al. 2011).

Air-polishing devices have been shown to be a safe and effective instrument in removing subgingival bacterial plaque around natural teeth (Flemmig et al. 2012; Petersilka et al. 2008). An original air-abrasive powder system has sodium bicarbonate as an abrasive powder for the removal of stain and plaque tooth surfaces (Weaks et al. 1984). A more recent abrasive powder, amino acid glycine, has lower abrasiveness to root cementum and dentin and still effective in removing dental plaque compared to sodium bicarbonate powder (Petersilka et al. 2003). It also has been shown that amino acid glycine powder does not result in specific surface alteration on titanium dental implant surfaces (Schwarz et al. 2009). While a recent systematic review showed that glycine powder air polishing is effective as the control treatment in peri-implant mucositis sites (Schwarz et al. 2015), only two prospective randomized controlled (Ji et al. 2014; Riben-Grundstrom et al. 2015) and one non-randomized controlled (De Siena et al. 2015) trials were included in the review. Moreover, most studies use a single application of air-polishing device in combination with other mechanical debridement instruments. Consequently, it is difficult to determine a true effect of an air-polishing device for the treatment of peri-implant mucositis.

Erythritol, a four-carbon alcohol sugar formed by the reduction of erythrose (Moon et al. 2010), has been used for repeated instrumentation of residual periodontal pockets during supportive periodontal therapy with higher patient's acceptance than scaling and root planing (Hagi et al. 2013) and ultrasonic debridement (Muller et al. 2014). Compared to glycine powder, erythritol powder has smaller particle sizes and slightly lower abrasiveness (Hagi et al. 2015). Both glycine and erythritol powder caused smallest gingival tissue damage compared to curets and ultrasonic scalers in an ex vivo model (Petersilka et al. 2018). Additionally, erythritol has inhibitory effects for biofilm development consisted of *Streptococcus gordonii* and *Pg* (Hashino et al. 2013). A combination of scaling and root planing with erythritol powder air-polishing device also showed an antimicrobial effect on *Pg* in a recent clinical trial (Park et al. 2018). Hence, the use of an erythritol powder air-polishing device could be beneficial for the treatment of peri-implant mucositis.

Peri-implant crevicular fluid levels of pro-inflammatory cytokines such as IL-1β have been used to assess reversibility after experimental peri-implant mucositis (Salvi et al. 2012). IL-1β has been suggested as one of major orchestrators of immune response after oral hygiene abstention and strongly correlated with gingivitis and peri-implant mucositis (Schincaglia et al. 2017). In this study, peri-implant crevicular fluid (PICF) will be analyzed for the semi

quantification of 27 inflammatory cytokines to evaluation peri-implant soft tissue condition and distinguish between healthy and diseased implants. The cytokines tested are Interleukin(IL) IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, IL-9, , Fibroblast growth factor (FGF), Granulocyte-macrophage colony-stimulating factor (GM-CSF), Granulocyte colony-stimulating factor (G-CSF), Macrophage inflammatory protein (MIP), regulated upon activation, normal T cell expressed and secreted (RANTES), Tumor necrosis factor (TNF), Platelet-derived growth factor-BB (PDGF-BB), Monocyte chemoattractant protein (MCP).

Despite the potential benefits of an air-polishing device with erythritol powder, clinical trials using the device as a monotherapy for treating patients with peri-implant mucositis remained limited. Therefore, the primary aim of the present study is to compare clinical outcomes of the non-surgical treatment of peri-implant mucositis and subsequent supportive peri-implant therapy (SPIT) using an erythritol air-polishing device or conventional mechanical debridement. The secondary aims are to compare the resolution of inflammation and amount of periodontal pathogens after non-surgical treatment of peri-implant mucositis and to monitor levels of peri-implant tissues inflammation during supportive peri-implant therapy by using the level of selected pro-inflammatory cytokines in PICF.

The titanium particles released during the implant placement was considered one of the trigger mechanisms for peri-implant bone loss, and further contribute to peri-implant complication (Fretwurst et al 2018). As a result, the effect of air-polishing treatment on implant surface should be carefully exanimated. In this study, the integrity of titanium implant will be examined by measuring the release of titanium particles after air-polishing.

II. Objectives

Primary objective: To compare clinical outcomes at baseline, 2 weeks, 4 weeks, 3 months, and 6 months after non-surgical treatment of peri-implant mucositis and effectiveness of supportive peri-implant therapy using an air-polishing device or conventional mechanical debridement.

- Null Hypothesis (H_0): No differences in clinical outcomes after non-surgical treatment of peri-implant mucositis between the erythritol air-polishing device treated and conventional mechanical debridement groups at any time points.

- Alternative Hypothesis (H_1): Clinical outcomes after non-surgical treatment of peri-implant mucositis are improved in implants treated with an erythritol air-polishing device compared to conventional mechanical debridement.

Secondary objective: To compare changes in the level of inflammatory cytokines in peri-implant crevicular fluid (PICF) at baseline, 2 weeks, 4 weeks, 3 months, and 6 months after non-surgical treatment of peri-implant mucositis using an erythritol air-polishing device or conventional mechanical debridement.

- Null Hypothesis (H_0): No differences in inflammatory cytokines in PICF after non-surgical treatment of peri-implant mucositis between the erythritol air-polishing device treated and conventional mechanical debridement groups at any time points.
- Alternative Hypothesis (H_1): Inflammatory cytokines in PICF after non-surgical treatment of peri-implant mucositis are higher in implants treated with conventional mechanical debridement compared to an erythritol air-polishing device.

Secondary objective: To compare changes in the level of bacterial DNA of *Aa, Pg, Pi, Tf,* and *Td* in PICF at baseline, 3 and 6 months after non-surgical treatment of peri-implant mucositis using an erythritol air-polishing device or conventional mechanical debridement.

- Null Hypothesis (H_0): No differences in *microbial community composition* in PICF after non-surgical treatment of peri-implant mucositis between the erythritol air-polishing device treated and conventional mechanical debridement groups at any time points.
- Alternative Hypothesis (H_1): *microbial community composition* in PICF after non-surgical treatment of peri-implant mucositis are different in implants treated with conventional mechanical debridement compared to an erythritol air-polishing device.

Secondary objective: To evaluate the influence of air-polishing therapy with erythritol on implant titanium surface.

- Null Hypothesis (H_0): No changes in physical property on titanium surface after air-polishing treatment
- Alternative Hypothesis (H_1): Air polishing treatment with erythritol powder will change the physical property of implant titanium surface.

Significance

Provide objective outcomes and better understanding on peri-implant tissue conditions after non-surgical treatment of peri-implant mucositis with subsequent SPIT which would help

determine gold standard protocols for peri-implant maintenance therapy for patients treated for peri-implant diseases.

III. Materials/Methods

Trial design

The design is a single blinded, randomized comparative effectiveness trial of a 6-month clinical intervention trial including two study groups with a diagnosis of peri-implant mucositis according to 2017 World Workshop (Berglundh et al. 2018a), and one group with healthy implants. 60 subjects will be recruited to allow for withdrawals and screening failures. There will be a total of at least 51 subjects (determined by the power calculation) completed for this study. Subjects must be a patient of record at the University of Michigan School of Dentistry. Subjects will be receiving non-surgical treatment and supportive peri-implant therapy at baseline, 3 months, and 6 months after baseline. PICF will be collected from the affected implant at baseline, 2 weeks, 4 weeks, 3 months and 6 months.

Study settings

This investigation will be conducted at the University of Michigan-School of Dentistry, Ann Arbor, Michigan, USA. Research subjects will be recruited from patients seeking and/or receiving dental care at the University of Michigan-School of Dentistry, Department of Periodontics and Oral Medicine, which also will serve as the coordinating center responsible for sample processing.

Subject Recruitment

1. *Recruitment/Pre-screening Examination*

Flyers will be displayed and dental providers at the University of Michigan-School of Dentistry will be notified that the study team is seeking subjects for this study. Each floor of the dental school has designated areas where flyers with information about current research projects that are recruiting patients are posted. These areas are clearly visible to dental school patients, dental students, dental hygiene students and faculty dental care providers. Recruitment will also consist of direct contact with dental students and faculty at the University of Michigan.

Co-investigators will review dental electronic health records (EHR) from potential patients scheduled for dental prophylaxis, periodontal maintenance, scaling and root planing, and/or surgical interventions. The study team may contact patients by telephone to see if they are interested in participating as a research patient.

2. *Consenting of Subjects*

Subjects will be consented in a private cubicle inside the Periodontics and Oral Medicine Department. A study team member will provide each potential study candidate with a written informed consent form (ICF) prior to enrolling the patient. Study personnel will explain to each patient and/or legal guardian/authorized representative the protocol, procedures and objectives of the study, and the patient's role in the study before obtaining consent. Subjects will be given the opportunity to read the informed consent. Study personnel will answer all questions that the subject may have and ensure that the subject understood all aspects of the study. When the subject (or legal guardian/authorized representative) understands and is willing to (have the subject) participate in the clinical trial, he/she (or the legal guardian/authorized representative) must sign and date the Institutional Review Board (IRB) approved Informed Consent Form. Subjects will be given a copy of the signed ICF for their records.

3. *Screening Examination*

This screening visit will consist of the following procedures:

- All subjects must have completed a medical health history questionnaire.
- Clinical measurements including probing pocket depth (PPD), recession (REC), bleeding on probing (BOP), plaque index (PI), and gingival index (GI) will be collected around the dental implant with peri-implant mucositis.
- Marginal bone loss (MBL) from periapical radiographs using calibrated digital measurements. Periapical radiographs will be obtained from periodontal records within the MiDent patient management system.

If requested by the subjects, information concerning the study and their participation will be shared with their healthcare provider (general dentist or physician). Study personnel will obtain consent from the subject to release all pertinent information (Appendix A). This study protocol will conform with the ethical guidelines of the 1975 Declaration of Helsinki as reflected in obtainment of approval by the University of Michigan's human subjects research review committee (IRB).

Patient selection

The CONSORT guidelines for clinical trials are followed (Fig.1)

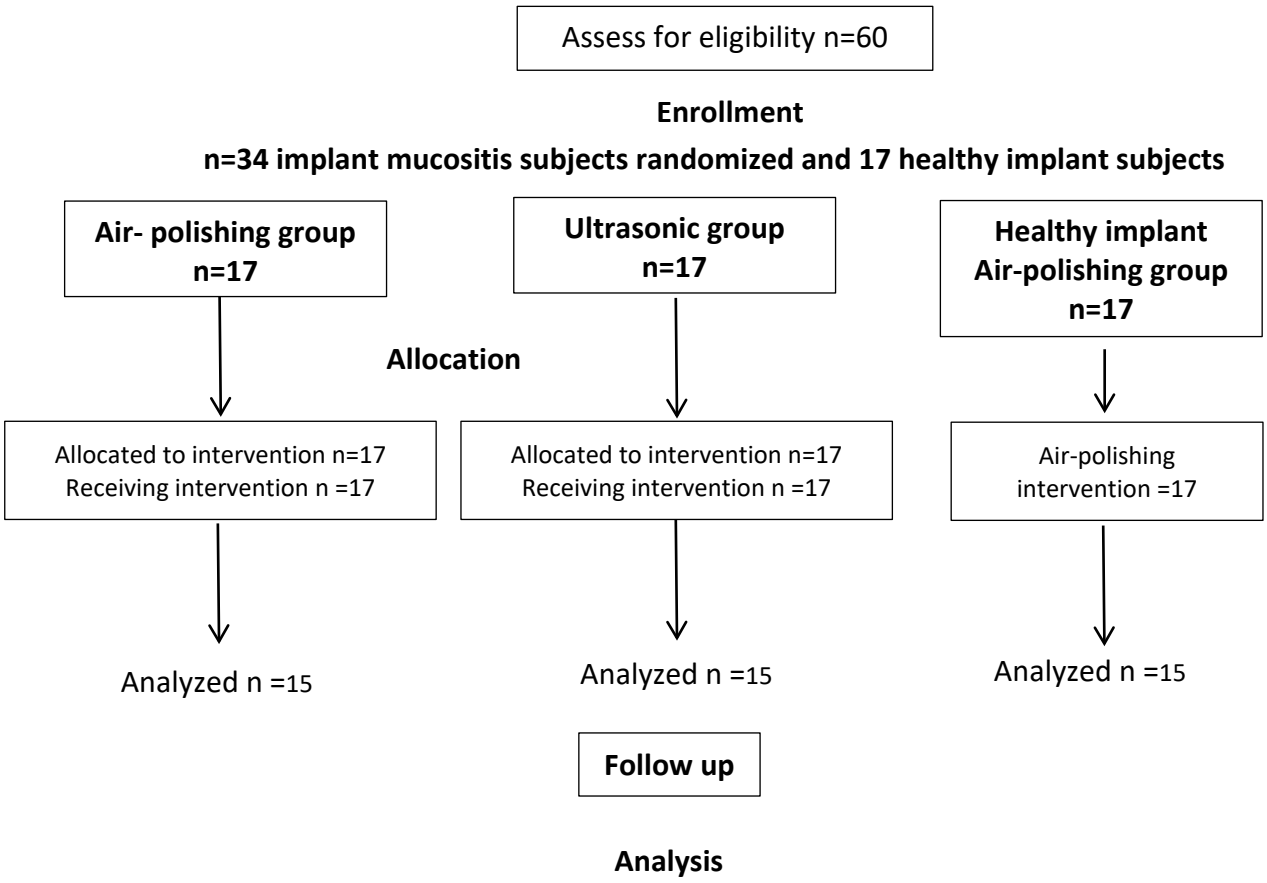
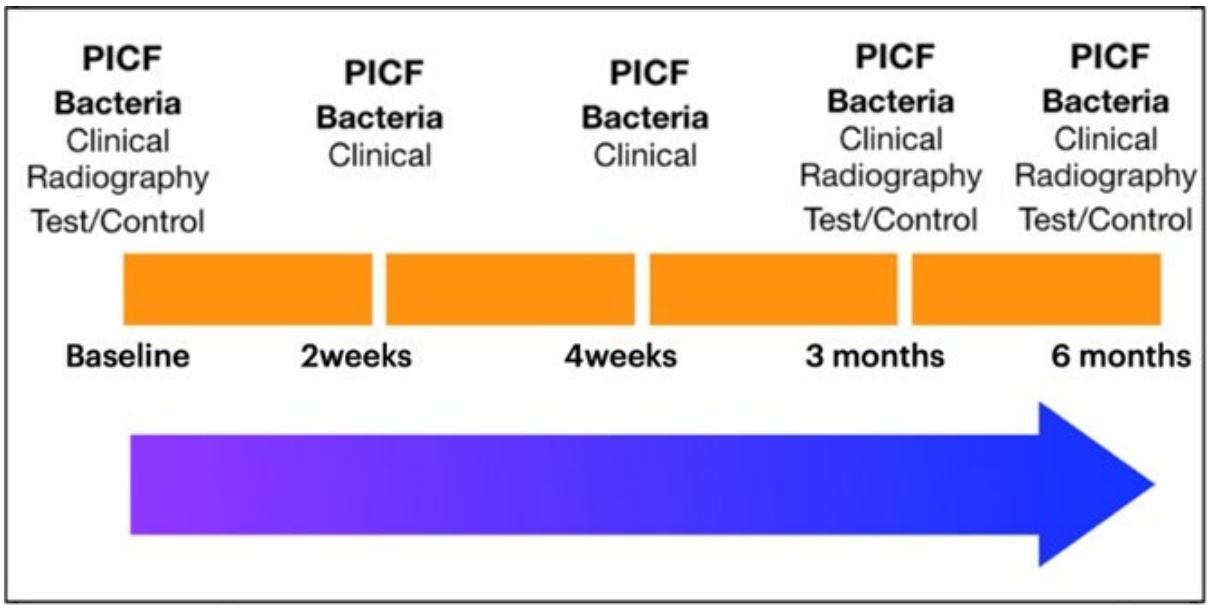


Fig1. CONSORT flow chart illustrating data collection and treatment delivery time points.



Eligibility

Inclusion criteria:

1) Age 18 to 85 years.
2) Healthy systemic conditions (American Society of Anesthesiologists classification (ASA) I or II: systemically healthy or suffer from mild to moderate, but well controlled systemic diseases).
3) Must have at least one osseointegrated dental implant with the following conditions (Berglundh et al. 2018a).
    - At least 12 months since the prosthesis was placed.
    - Absence of bone loss beyond crestal bone level changes from initial bone remodeling.
    - For implant mucositis subjects: Presence of bleeding and/or suppuration on gentle probing around implant at least 2 sites. Visual inspection demonstrating the presence of peri-implant signs of inflammation.
    - For healthy implant subjects: Absence of bleeding and/or suppuration on gentle probing around implant.
4) Have a current (less than 6 months old) x-ray of the affected site
5) All participants must be enrolled in a 3 to 6 months maintenance recall program including teeth/implants prophylaxis and supportive periodontal therapy.

Exclusion criteria:

1) Poorly controlled diabetes mellitus (HbA1c >6.5).
2) Uncontrolled systemic disease or condition known to alter bone metabolism. (e.g. osteoporosis, osteopenia, hyperparathyroidism, Paget's disease)
3) Current smokers or vapers.
4) Pregnancy or could be pregnant (self-reported), or plan to be pregnant in the next 6 months.
5) Long-term (3 months) intake of anti-inflammatory medications (e.g. non-steroidal anti-inflammatory drugs (NSAIDs)) known to affect periodontal status within one month previous to participation in the study.
6) Taking corticosteroids.
7) Long-term antibiotic use (> one weeks) within three months previous to participation in the study.

8) No history of supragingival scaling or non-surgical therapy at the affected implant site in the last 1 month.
9) Taking anticoagulant medications.
10) Subjects who require prophylactic antibiotics.

Randomization

The randomization will be performed using a computer software program (SPSS Inc., Chicago, IL, USA). The subjects will be sequenced to the therapy allocated by a clinician who is not involved with the study. The subjects will be informed not to discuss the therapy with the examiner. The study examiner will be blinded of treatment allocation and evaluate all clinical measurements.

PICF and Subgingival plaque biofilm collection

Supragingival plaque will be first removed with sterile cotton pellets. The site will be isolated and air dried. Subgingival plaque will be collected at the deepest site of the affected implant. At least 10 sterile paper points will be inserted apically until resistance was felt at the base of the peri-implant sulcus for plaque sampling and left for 30 seconds. The paper points will be pooled and immediately placed into labeled vials containing 500 µl of stabilizing buffer (RNAprotect™, Ambion, Austin, TX, USA) and shaken for 10 seconds. The samples will be vortexed for 30 seconds and placed on dry ice for transport to the laboratory and stored at -80 ˚C. The PICF sample will be collected from the same site using 6 PerioPaper® (Oraflow Inc., Smithtown, NY, USA) strips gently inserted into the peri-implant crevice until light resistance is felt and left for 30 seconds. The volume of fluid collected will be measured using a Periotron8000 (OraFlow Inc.). The strips will be kept separately in each individual 1.5 ml Eppendorf tube at -80 ˚C.

Sample transportation, storage and processing

For the analysis of PICF samples, a 20µl extraction solution containing 10µg/ml aprotinin, 1mM phenylmethylsulfonyl fluoride, and 0.1% human serum albumin in phosphate-buffered saline (PBS, pH 7.4) will be pipetted directly onto the cellulose portion of each PerioPaper® strip and secured at the top of a 12x75mm polystyrene culture tube using a cap to hold it in place. After centrifugation at 2,000 rpm at 40C for 5 min, each strip will be washed five times to yield a total elution volume of 100µl. The samples will be stored at -80°C.

Quantitative assessments of biomarker expression in PICF samples will be performed using custom human Quantibody® arrays.

For the analysis of plaque samples, the plaque absorbed onto paper points will be vortexed in Eppendorf tubes® (Eppendorf AG, Hamburg, Germany) to suspend the plaque. The paper points will be removed, and the tubes will be centrifuged to pellet bacteria. Total DNA from the plaque sample will be purified using the MasterPure™ Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) according to the instructions of the manufacturer.

Laboratory analysis

1. Microbial analysis

DNA will be isolated as previously described (Kumar et al. 2011) and multiplexed bacterial tag-encoded FLX 16S pyrosequencing will be performed using the Illumina platform. Two regions of the 16S rRNA genes will be sequenced: V1–V3 (spanning *E.coli* 16S gene regions 8-27 and 519-536) and V7–V9 (spanning *E.coli* 16S gene regions 1099-1114 and 1528-1541). The primers used for sequencing have been previously described (Kumar et al. 2011). Sequences with an average quality score of 30 over a sliding window of 50bp and length >200 bp will be assigned a taxonomic identity by alignment to the HOMD database (Chen et al. 2010) using the Blastn algorithm. Analyses will be conducted using the QIIME (Caporaso et al. 2010) and PhyloToAST (Dabdoub et al. 2016) pipelines.

2. Cytokine analysis

PerioPaper strips will be first thawed on ice and PICF was eluted with 200 µl of elution buffer containing 50mM Tris/HCl with 5mM $CaCl_2$, 0.2 NaCl pH 7.6, 1mg/L antipain, 1 mg/L aprotinin, 1 mg/L leupeptin, 125 mg N-ethylaleimide and 50 mg Zwittergent 3-12. The sample will vortex vigorously at 15-minute intervals for an hour. Cytokine analysis was done using a commercially available mutiplexed bead-based assay allowed to quantitate multiple cytokines. 27 distinct sets of fluorescently dyed beads (Bio-Plex Pro™ Human Cytokine Panel) will be conjugated with monoclonal antibodies specific for each cytokine to be tested and incubated with 50µl of GCF. 25 µl of biotinylated detection antibody will be added and 50µl of Streptavidin- phycoerythrin reporter will be sequentially added. The level of each cytokine will be assessed using a Bio-Plex 200 system and will be measured by comparison to a standard curve.

3. Mass Spectrometry Assays for titanium detection

The protocols used were modified from Safioti et al., (2017). LDPE tube will be washed three times with de-ionized water before use. The acid mixture with HNO3 (trace metal grade) and HF (trace metal grade) will be prepared. The samples will be transferred to the LDPE tube and 85 µl of acid mixture will be added. Another 550 µl deionized water will be added for a total volume of 685 µl. Positive controls will be POBS and negative controls with de-ionized water will be prepared using acid mixture and de-ionized water. Indium (12.5 ppb) will be used as the internal control. The samples will be run in the Inductively Coupled Plasma – Mass Spectrometry machine (Thermo Finnigan Element 2, USA). The take-up time will be set at 50 seconds. The machine will be calibrated with internal standards containing known 24 concentrations of Titanium (0.01 ppb, 0.5 ppb, 1 ppb, 10 ppb and 50 ppb). In addition, the internal standards were repeated for every 8-10 samples to ensure internal validity. The concentration of Titanium 49 and Titanium 47, 49, 50 will be noted and, calculations will be performed using the dilution factors to estimate the amount of Titanium in the sample in ppb. The person conducting the ICP-MS experiments will be blinded and no clinical information was available to researcher who performed the experiments and calculated the concentrations.

Examiner calibration

The clinical examination will be performed by multiple calibrated examiners using the kappa statistic (0.76) as a reference for calibration.

Clinical measurements

The probing depth (PD) and bleeding on probing (BOP) will be measured by using a plastic probe (UNC COLORVUE™ probe, Hu-Friedy, Chicago, IL, USA). Clinical measurements will include (I) full-mouth plaque score using disclosing dye and record the presence or absence of dental plaque along marginal gingiva/mucosa as a percentage of examined sites (four sites per tooth/implant); (II) local plaque score at six sites per implant, (III) local PD at six sites per implant; (IV) mucosal recession (REC) relative to the restoration margin at the implants at two sites (buccal or lingual/palatal); (V) tissue thickness will be measure by probe visibility (Rasperini et al. 2015); (VI) modified gingival index (GI) (Loe 1967) at each of the four mucosal area of the implant will be scored as (0) normal peri-implant mucosa, (1) slight change in color and mild edema without bleeding in probing, (2) redness, edema and bleeding on probing and (3) marked redness and edema with tendency to spontaneous bleeding; (VII) full-mouth bleeding scores and present as a percentage of

examined sites (four sites per tooth/implant); (VIII) local presence of absence of BOP at the implant at six sites per implant; (IX) amount of bleeding at the implant sites will be scored as (0) no bleeding, (1) pin-point bleeding, (2) line of bleeding and (3) drop of bleeding along mucosal margin on gentle probing (Mombelli et al. 1987) at six sites per implant; and (X) presence or absence of exudate. All clinical measurements will be performed at baseline, 2 weeks, 4 weeks, 3 months, and 6 months. Standardized intraoral periapical radiographs will be obtained at baseline, 3 months, and 6 months using sensor holders with bite registration to obtain reproducible radiographs and to detect loss of supporting bone due to allocated intervention.

Non-surgical treatment protocol

For concealment of allocation, the type of treatment in a sealed envelope will be given to the clinician before providing non-surgical treatment. The implants in the Air-polishing group will be treated using the air- polishing device (AIR-FLOW HANDY 3.0 Premium, Hu-Friedy, Chicago, IL, USA) with an erythritol powder (Air-Flow Plus, Hu-Friedy, Chicago, IL, USA). The nozzle will be placed in the peri-implant pocket at an angle of 45-60 degrees to the implant and each surface will be blasted for 30 seconds. In the conventional group, each surface of the implants will be debrided for 30 seconds with an ultrasonic device with plastic tip (NSK Varios 350 and V-P10, Varios, NSK, IL, USA). Treatment time will be recorded by a digital timer during treatment of both groups.

At every 3-month interval, an implant examination will be completed, individualized oral hygiene instruction will be reinforced, and professional cleaning will be performed. In the Air-polishing group, the implants will be blasted with an erythritol air-polishing device while implants in conventional group will be cleaned by an ultrasonic device with plastic tip. Supragingival plaque will be removed with rubber cup and low-abrasive polishing paste.

Patient's tolerance evaluation

A visual analog scale (VAS) assessing the patient's tolerance perception during treatment will be given immediately after completion of treatment during the first (BL) and last study (6 month) visits. The patient's perception will be evaluated by a subjective VAS, with score 0 representing the most comfortable or no pain and 10 representing the most uncomfortable or the worst pain that the patient has ever experienced during treatment (Appendix B). Subjects' responses (where they marked an X on the line) will be measured with a ruler to quantify their score between 0 and 10.

Schedule of Events

Visit	Timeline	Procedures
1	Baseline (BL)	• Explanation of the study and inform consent completion • History taking (dental history, medical history) • Initial oral examination • PICF and bacterial sample collection from the affected implant • Implant examination (PD, REC, plaque scores, GI, BOP) • Intraoral photographs of the affected implant • Bite registration on sensor holder • Periapical radiograph of the affected implant • Periodontal examination (Full-mouth PPD, plaque scores, GI, BOP) • Non-surgical therapy of peri-implant mucositis (test: an air-polishing device, control: an ultrasonic device with plastic tip) • Complete VAS score questionnaire
2	2 weeks (+/- 1weeks) from BL	• Review medical history/adverse events • PICF and bacterial sample collection from the treated implant • Implant examination (PD, REC, plaque scores, GI, BOP) • Intraoral photographs of the treated implant
3	4 weeks (+/- 1 weeks) from BL	• Review medical history/adverse events • PICF and bacterial sample collection from the treated implant • Implant examination (PD, REC, plaque scores, GI, BOP) • Intraoral photographs of the treated implant •
4	3 months (+/- 2 weeks) from BL	• Review medical history/adverse events • PICF and bacterial sample collection from the treated implant • Implant examination (PD, REC, plaque scores, GI, BOP) • Intraoral photographs of the treated implant • Periapical radiograph of the treated implant • Supportive peri-implant maintenance therapy (test: an air-polishing device, control: an ultrasonic device with plastic tip)
5	6 months (+/- 2 weeks) from BL	• Review medical history/adverse events • PICF and bacterial collection from the treated implant • Implant examination (PD, REC, plaque scores, GI, BOP) • Intraoral photographs of the treated implant • Supportive peri-implant maintenance therapy (test: an air-polishing device, control: an ultrasonic device with plastic tip) • Periapical radiograph of the treated implants • Complete VAS score questionnaire

IV. Statistical analysis

Sample size calculation

A minimum of 17 patients per treatment is required to have 80% power of detecting the difference of 0.6mm of PD between groups with statistically significant α=0.05 (De Siena et al. 2015).

Statistical methods

The sample demographic data will be analyzed using repeated univariate analysis of variance for adjusting the subject factor including the number of implants in each subject.

Clinical parameters for the two groups will be calculated for mean values and standard deviation. Analyses of the investigated variables at every time point will be performed using Linear mixed model. Comparisons within group between baseline and every time point will be analyzed using paired t-test. Comparisons of differences between groups will be performed using Difference in differences analysis. Results will be considered as statistically significant at $p < 0.05$.

Biomarker levels and amount of the targeted bacterial changes over time will be analyzed using Linear mixed model. Comparisons between two groups will be performed using paired t-test. Correlations between biomarker levels, amount of the targeted bacteria levels, and clinical parameters will be analyzed by the Spearman rank order correlation test. All non-parametric data will be illustrated as medians and interquartile ranges in the box plot graphs.

V. Compensation

Compensation will be provided for all participants with up to two prophylaxes or periodontal maintenances with a reduced fee of 100% from original price at the Graduate Periodontics clinic at the University of Michigan-School of Dentistry.

VI. Study reporting

1. Adverse events

Overall risks for participation are very minimal. A slight discomfort after non-surgical treatment is expected.

ANY SERIOUS AND UNEXPECTED ADVERSE EVENT INCLUDING HOSPITALIZATON OR DEATH DUE TO ANY CASE, WHICH OCCURS DURING THIS INVESTIGATION, WHETHER OR NOT RELATED TO THE STUDY, MUST BE

REPORTED IMMEDIATELY (WITHIN 24 HOURS) TO THE PRINCIPAL INVESTIGATOR.

Reports of serious or unexpected adverse events will be made immediately by telephone or fax to: Alice Ou, Tel: 734-763-3346, E-mail: aliceou@umich.edu or Dr. Hom-Lay Wang, Tel: 734-763-3325, E-mail: homlay@umich.edu.

This e-mail report or fax must be followed within 5 days by a written summary fully documenting the event in order to permit the Principal Investigator to file a report, which satisfies regulatory guidelines.

All serious and unexpected adverse events associated with the use of the study test material will be reported immediately to appropriate regulatory agencies by the Principal Investigator.

Adverse events reporting will proceed according to the University of Michigan guidelines for standard AE reporting.

2. Discontinuation of subjects

- Any subject found to have entered the study in violation of this protocol will be withdrawn from the study after discussion with the Principal Investigator.
- An effort will be made to determine why any subject discontinues the study prematurely. This information should be recorded on the appropriate case report form (CRF).
- As stated in the informed consent, all subjects reserve the right to withdraw from the study at any time if they declined to run the planned tests of their samples.
- Any female subject that becomes pregnant between the screening and biopsy visits will be withdrawn from the study.
- Any subject, whose condition changes after entering the study, so that he or she no longer meets the inclusion or exclusion criteria, will be withdrawn from the study.
- Any subject who requires the use of an unacceptable concomitant medication will be withdrawn from the study.
- Subjects discontinued for adverse events will not be replaced.

3. Data reporting and case report forms (CRF)

Data reflecting participant experience with the protocol under investigation will be reported to the Principal Investigator and the data recorded on Case Report Forms (CRF).

CRF will be signed and dated by the investigator or a designated representative and filled out in black ink. If an entry on a CRF requires change, the correction will be made as follows:

a. A single line will be drawn through the incorrect entry.

b. The date will be entered and the change initialed. White-out or erasure on CRFs will not be permitted under any circumstance.

All fields and blanks must be completed. The following abbreviations will be used when values or answers cannot be provided: NA=not applicable; ND=not done, UNK=not known

Completed original CRFs will be collected by the Principal Investigator or Study Coordinator. CRF must be submitted for each subject.

Data entry will proceed directly from the CRF. The data, as well as group/subject identification, will be made available to the investigator at the conclusion of the study.

4. Study monitoring

The investigator or designated representative periodically will audit all CRFs and corresponding portions of School of Dentistry records. The monitoring will provide the Principal Investigator the opportunity to evaluate the progress of the study and to verify the accuracy and completeness of the CRFs; assure that all protocol requirements, applicable FDA regulations and investigator's obligations are being fulfilled, and to resolve any inconsistencies in the study records. The Principal Investigator may stop the study if it is observed that the protocol or sound clinical practices are not being followed. The Principal Investigator may exclude subjects from the study if review of their records indicates violations of the protocol or if there were other reasons to believe that their inclusion would jeopardize the validity of the study.

VII. Regulatory Considerations

1. Institutional Review Board (IRB)

The protocol for this study must be reviewed and approved by an appropriate IRB before tissue collection for the study.

It is the responsibility of the investigator to assure that all aspects of the institutional review are conducted in accordance with current Federal Regulations. A letter with IRB approval for the protocol must be received by the Principal Investigator before the initiation of the study. Amendments to the protocol will be subject to the same requirements as the original

protocol. At each annual protocol renewal, an updated report of the numbers of samples and any adverse events will be provided to the IRB.

After completion or termination of the study, the investigator will submit a final report to the IRB. This report should include any deviations from the protocol, the number of samples evaluated, the number of samples that were discarded including reasons, results of the study, adverse events, and a conclusion summarizing the results.

2. Informed Consent

A signed, written informed consent must be obtained from each subject before he or she enters the study after he or she has been given verbal and written information describing the nature and duration of the study. Subjects are not to be treated until an informed consent has been obtained. The signed informed consent will be retained with the study records. Each subject will also be given a copy of his/her informed consent.

As long as patient information is kept within the University of Michigan, it will be protected by the Health System's privacy policies. Information about these policies are available at http://www.med.umich.edu/hipaa/npp.htm. Patient information will be protected according to the privacy regulations of the Federal Health Insurance Portability and Accountability Act of 1996 (HIPAA).

3. Access of Records

The investigator understands that the office and hospital records of subjects entered in this study will be required to be available under the supervision of the investigator or a designated representative for inspection by the FDA. All subject related information provided to the FDA will be done so without subject names or other identifying information.

4. Retention of Data

The investigator will maintain adequate records for the study including participant's CRF, medical records, informed consent forms, safety reports, information regarding participants who discontinued, and any other pertinent data. All records will be maintained in a locked storage room to which only study investigators have access.

These records will be available to copying and inspection if requested by a properly authorized employee of the Department of Health and Human Services, under the supervision of the investigator or a designated representative and in accordance with federal regulations.

5. Deviation from the Protocol

The investigator will not deviate from the protocol without obtaining written approval from the IRB. Any other changes or deviation in the protocol will be made as an amendment to the protocol and must be approved by the IRB before being implemented.

6. Reports

The investigator will make an accurate and adequate written progress reports to the IRB at appropriate intervals not exceeding one year.

The investigator will make an accurate and adequate final report to the IRB within 3 months after completion or termination of the study.

The investigator will make an accurate and adequate special report to the IRB on any serious unexpected or life-threatening adverse event or death occurring in relationship to the study whether regarded as study-related or not.

On completion of the study, the investigators will prepare a final report of the study results.

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Appendix A

Consent to Release Information to the Subject's Dentist

Patient's Initials/Number: _____ Date: _____

Title of Study: Treatment of peri-implant mucositis and supportive peri-implant therapy using an erythritol air- polishing device versus conventional mechanical debridement. A 6-month randomized controlled clinical trial.

Principal Investigator:

Hom-Lay Wang, DDS, MS, PhD (Faculty Advisor)

Study site:

Graduate Periodontics, University of Michigan - School of Dentistry

1011 North University Avenue

48109-1078, Ann Arbor, MI – USA

CONSENT OF THE SUBJECT

By signing this document, you are agreeing that we inform your general dentist that you are participating in this study. Information regarding the objectives of the present study will be shared. This information will be given by mail or by phone. You will be given a copy of this document for your records and one copy will be kept with the study records. Be sure that all your questions concerning the study have been answered and understood.

I have read of the information of the information given above. I understand the meaning of this information. I hereby voluntarily consent to release my information to my general dentist.

_____

Name of Adult Subject of Research (Print)

_____ _____

Signature of Adult Subject of Research **Date**

_____ _____

Study Investigator/Coordinator Signature **Date**

Name of dentist: _____

Address: _____

_____

Phone number: _____

Appendix B

Post-Operative Patient Tolerance of the Treated Peri-implant Mucositis Site

Subject ID # _____ **Date**: _____

Please answer a following question. We want to assure you that all your answers are strictly confidential. Your name will not appear on any material connected with the data.

After having your teeth cleaned, please write an X on the line shown below.

How do you feel during the treatment you received? Score 0 representing the most comfortable or no pain and 10 representing the most uncomfortable or the worst pain you have ever received during treatment.

Score 0
Most comfortable
No pain

Score 10
Most uncomfortable
Worst pain I've ever experienced