Distribution Statement

Distribution A: Public Release.

The views presented here are those of the author and are not to be construed as official or reflecting the views of the Uniformed Services University of the Health Sciences, the Department of Defense or the U.S. Government.

**UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES**
POSTGRADUATE DENTAL COLLEGE
NAVAL POSTGRADUATE DENTAL SCHOOL
8955 WOOD ROAD
BETHESDA, MARYLAND 20889

THESIS APPROVAL PAGE FOR MASTER OF SCIENCE IN ORAL BIOLOGY

Title of Thesis: Assessment of pH around healthy and questionable titanium dental implants

Name of Candidate: Alexandra L. Doak
Master of Science Degree
April 14, 2023

THESIS/MANUSCRIPT APPROVED: DATE:

KIM.JEFFREY.J.1553853377 Digitally signed by KIM.JEFFREY.J.1553853377 Date: 2023.06.05 13:07:14 -04'00' 6/5/23

Jeffrey J. Kim
RESEARCH DEPARTMENT
NAVAL POSTGRADUATE DENTAL SCHOOL
Committee Chairperson

HERNANDEZFELDPAUSCH.MARINA.1300102960 Digitally signed by HERNANDEZFELDPAUSCH.MARINA.1300102960 Date: 2023.06.13 16:46:02 -04'00' 6/13/23

Marina Hernandez-Feldpausch
COMPREHENSIVE DENTISTRY DEPARTMENT
NAVAL POSTGRADUATE DENTAL SCHOOL
Committee Member

DARCEY.CAITLIN.DORA.1365197101 Digitally signed by DARCEY.CAITLIN.DORA.1365197101 Date: 2023.06.14 08:15:20 -04'00' 6/14/23

Caitlin D. Darcey
PERIODONTICS DEPARTMENT
NAVAL POSTGRADUATE DENTAL SCHOOL
Committee Member

# ASSESSMENT OF PH AROUND HEALTHY AND QUESTIONABLE TITANIUM DENTAL IMPLANTS

by

Alexandra L. Doak
Lieutenant Commander, Dental Corps
United States Navy

A thesis submitted to the Faculty of the
Comprehensive Dentistry Graduate Program
Naval Postgraduate Dental School
Uniformed Services University of the Health Sciences
In partial fulfillment of the requirements for the degree of
Master of Science
in Oral Biology
June 2023

**THESIS APPROVAL PAGE**

**INTENTIONALLY LEFT BLANK**

# ACKNOWLEDGMENTS

I would like to thank my research committee and especially Dr. Kim for support and assistance in completing this project. Thank you to my colleagues in the Comprehensive Dentistry department for help in identifying potential research subjects.

# DEDICATION

To my husband, Kyle, for your encouragement. And most importantly to my son, Jack. He is too young to know about work and stress, but everything I do is for him.

# DISCLAIMER

The views presented here are those of the author and are not to be construed as official or reflecting the views of the Uniformed Services University of the Health Sciences, the Department of Defense or the U.S. Government.

# ABSTRACT

Assessment of pH Around Healthy and Questionable Titanium Dental Implants

Alexandra Laurann Doak, DDS, 2023

Thesis directed by: Jeffrey J. Kim, DDS, PhD
Department Chair
Research Department
Naval Postgraduate Dental School

**Introduction**: Peri-implant mucositis and peri-implantitis is currently diagnosed using probing depths, bleeding on probing and radiographic bone loss. The subjectivity of these parameters can make accurate diagnosis challenging. Prognosis of implant related diseases is even more difficult. The use of another physiological factor, such as pH of peri-implant crevicular fluid, could aid in identification of ailing implants. **Objective**: The purpose of this study was to determine a correlation between pH and implant health. Crevicular fluid surrounding healthy and ailing implants was compared to the fluid surrounding healthy, natural teeth. **Methods**: The implants were identified as healthy or ailing based on observations such as probing depths, bleeding on probing, inflammation, infection, and radiographic bone loss. Gingival fluid collection strips (Periopaper Pro Flow™) were placed within the sulcus surrounding the implant and the contralateral natural tooth (control). These strips were left in place until fluid saturation. Fluid was extracted from the strips in a 1.8 mL micro-tube following centrifugation for 2 cycles of 10 minutes (4°C and 15,000 RPMs). Samples were stored

in a -80°C freezer until testing. pH of the fluid was measured using a micro-pH probe. The mean pH of the samples of implants and natural teeth were compared among subjects. **Results**: Fifteen human subjects were enrolled with two ailing and thirteen healthy implants. The mean pH of healthy implants was 6.30 ± 0.62 and the mean pH of contralateral natural teeth (control) was 6.56 ± 1.14. pH of the ailing implant was 3.76 and pH of healthy control tooth from the same subject was 6.21. **Conclusions**: A tentative positive correlation was observed from pH and status of the dental implant with an ailing implant having a lower pH compared to healthy implants. Within the same subject, the pH was similar to the pH surrounding dental implants. Subjects will continue to be enrolled.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF ABBREVIATONS

| | |
|---|---|
| BOP | Bleeding on probing |
| DoD | Department of Defense |
| GCF | Gingival crevicular fluid |
| NPDS | Naval Postgraduate Dental School |
| PICF | Peri-implant crevicular fluid |
| WRNMMC | Walter Reed National Military Medical Center |

# CHAPTER 1: Introduction

## BACKGROUND

The 2017 World Workshop introduced specific definitions of implant health and disease to help guide practitioners in diagnosis and management of peri-implant conditions.[1] Implants can fail from esthetic, mechanical, and biological complications. Biologic complications of implants are inflammatory conditions of the soft tissue and bone induced by the accumulation of bacterial biofilm.[2] These conditions include peri-implant mucositis and peri-implantitis. In the absence of clinical signs of inflammation there is peri-implant health. The mucosa should form a tight seal around the implant and restorative components, and there should be no bleeding on probing or bone loss following initial healing.[2] Peri-implant mucositis is an implant disease that includes soft tissue inflammation without additional bone loss following initial healing. Peri-implantitis is inflammation affecting the hard and soft tissues around a functioning osseointegrated implant with progressive loss of supporting bone.[3] The etiology of both implant inflammatory conditions is multifactorial, including the bacterial biofilm, prosthetic and patient related factors.[4] To differentiate peri-implant health from diseased conditions, there should be no visual signs of inflammation, lack of profuse bleeding on probing, probing depths less than or equal to 5mm, and absence of bone loss following initial healing not to exceed 2mm.[2] If implant evaluation exceeds these diagnostic criteria for implant health, a diagnosis of peri-implant mucositis or implantitis will be made. After the 2017 World Workshop, there is a consensus on diagnostic criteria for implant health and disease.[1] Radiographic signs of progressive bone loss can be quantifiably measured, and signs of pain,

mobility, and suppuration can be clinically evaluated. However, due to the absence of a periodontal ligament around an implant as well as prosthetic design, it is challenging to perform and interpret probing depth measurements. There is less resistance to probing soft tissue around an implant in comparison to adjacent natural teeth.[5] This means that providers may inadvertently probe with greater force, inducing bleeding on probing, than they would around natural teeth causing a traumatic episode versus a pathologic response. As bleeding on probing and probing depths are clinical criteria used to differentiate peri-implant health from peri-implant mucositis, diagnosis could be aided by use of another criteria. A pilot study by Nyako et al in 2005 tested the pH of peri-implant crevicular fluid (PICF) of failing and successful implants.[6] They found that the PICF of failing implants had a mean pH of 7.20 compared to 6.80 for successful implants. Failing implants were defined as exhibiting peri-implant alveolar bone loss over time. Review of the literature showed that there are minimal studies evaluating pH of peri-implant soft tissue. There are multiple studies evaluating the microbial profile of PICF of healthy and failing implants. Inflammatory mediators like Interleukin-1B and TNF-alpha in PICF can be used to assist in early diagnosis of peri-implant diseases.[7]

The purpose of this study is to measure the pH of peri-implant crevicular fluid around healthy and questionable implants in comparison to natural teeth. If a difference is found, pH values could be used as an adjunct tool to assess the status of dental implants.

# CHAPTER 2: Materials and Methods

## STUDY DESIGN

Walter Reed National Military Medical Center (WRNMMC) Institutional Review Board approved this study (WRNMMC-2022-0381).

The aim of this descriptive study was to establish a baseline pH of healthy teeth and implants and determine if there is a correlation between the health status of the implant and the corresponding PICF pH.

Potential research subjects with dental implants of interest were recruited from dental treatment facilities at NPDS. After the study was carefully explained, informed consent was presented and obtained. An initial examination was completed, and the clinical data were collected. Implant health status was recorded as healthy or ailing. For the purposes of this study, both peri-mucositis and peri-implantitis were assigned to the category of "ailing" implants to describe implants that were in a diseased state and require clinical interventions.

## SUBJECT INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria included at least one dental implant to serve as the test site and a contralateral tooth or implant available to serve as baseline control. The ideal baseline control was a contralateral tooth or implant at a "mirror" location (e.g., left lower first molar implant paired with right lower first molar tooth). However, one location forward or reverse was accepted as needed (e.g., left upper second molar implant paired with right upper first molar tooth). The exclusion criteria included subjects with a failing implant

requiring immediate intervention, rampant caries, severe periodontal disease requiring immediate attention, severe xerostomia, uncontrolled systemic disease, or other dental emergencies requiring immediate attention.

### DATA AND SAMPLE COLLECTION

A master data collection sheet was compiled with the following clinical information: implant location, health status of the implant (healthy versus ailing), bone loss around the implant (only if radiographs exist), presence and periodontal status of the contralateral tooth/implant. Later, during lab processing, the pH of PICF and GCF samples were recorded and added to the master data collection sheet.

The GCF/PICF collection process is depicted in the flowchart shown in **Figure 1**. Gingival fluid collection strips, Periopaper (Pro Flow™), were used to collect the GCF/PICF from the subjects **(Figure 1A)**. The collection strips were placed into the gingival sulcus around the identified implant and contralateral tooth/implant (baseline control) and left in place for approximately twenty seconds **(Figure 1C)**. This process was repeated 2-3 times depending on how much fluid was visible on the strip. The minimum threshold was to collect approximately 10 µL of GCF/PICF around each tooth/implant. The collection strips were placed in a 1.8 mL micro-tube with a modified insert to allow for a 2-stage collection, so the paper and GCF/PICF remained separated after centrifuge **(Figure 1D)**. The samples were then transferred on ice and placed in a centrifuge for 2 cycles of 10 minutes at 15,000 RPM / 4°C to isolate the GCF/PICF **(Figure 1E)**. The samples were placed on ice again and transferred to a -80°C freezer. Once the collection phase of the study was complete, all samples were removed from the

freezer and a micro-pH meter was used to measure the pH of the GCF/PICF **(Figure 1F)**. This allowed for all samples to be measured with the same calibration of the pH meter. The pH of samples was recorded on the master data collection sheet.

# CHAPTER 3: Results

Fifteen subjects were enrolled in the study. All subjects had received a periodontal diagnosis regarding their implant and the contralateral tooth prior to participation in the study. Radiographs were viewed by the principal investigator to confirm diagnoses. Two subjects with ailing implants and thirteen subjects with healthy implants were identified. The results are summarized in **Table 1**. pH results from two subjects (one healthy and one ailing implant) could not be assessed due to insufficient GCF and PICF collection from the paper collection strips. This is reflected in the tables. The pH of the ailing implant was 3.76 and its contralateral tooth was 6.21. The mean pH of the healthy implants was $6.30 \pm 0.62$ and the mean pH of the healthy control contralateral teeth was $6.56 \pm 1.14$. **Figure 2** shows a graphical representation comparing the ailing implant with the mean of the healthy implants. The pH of the measurable ailing implant was lower than the mean pH of the healthy implants and contralateral teeth of all implants as shown in **Figure 3**.

# CHAPTER 4: Discussion

The goal of this study was to assess the feasibility of our method and to measure the baseline pH of ailing and healthy implants and their contralateral teeth. Gingival fluid collection strips were originally designed for other purposes; however, we found that enough fluid could be collected to determine pH. Previously, pH measurements have been performed using an electrode which was directly inserted into the gingival sulcus space. Because pH electrodes are made of glass and have sharp ends, they pose substantial safety risks to human subjects (i.e., glass electrode breaking inside of sulcus). Gingival fluid collection strips provide a safer way to collect fluids and measure pH.

Of the fifteen subjects, two subjects were identified as having ailing implants; however, the pH from only one of these subjects could be measured. Thirteen subjects were identified as having healthy implants, and the pH from twelve of these implants could be measured. The minimum fluid volume needed to reliably measure pH is 5 μL. The findings of this study found that the mean pH for healthy implants was 6.30, which corroborates with previous studies that reported the mean pH measurements to be 6.80, 6.64, and 6.90.[6,8,9] The results indicate that the ailing implant had a lower pH in comparison to healthy implants and all contralateral natural teeth. This finding differs from previous studies that reported that GCF in sites of inflammation in natural teeth and overall failing implants showed an increased pH as compared to healthy sites.[6,8] The discrepancy between our findings and previous publications may be due to the severity or chronicity of the peri-implantitis the patient presents with. There is a spectrum between healthy and diseased periodontia. As such, a similar spectrum exists in implants (i.e., a

healthy implant, acute and chronic peri-implantitis of the ailing implant, and a failed implant). During the early stages of microbial colonization of the periodontal pocket, there is a shift towards a more acidic GCF due to *Fusobacterium nucleatum* and *Prevotella intermedia,* which metabolize carbohydrates.[10] As gingival inflammation progresses and pocket depth increases, pH of the GCF becomes less acidic due to colonization by *Porphyromonas gingivalis*, proteolytic bacteria that break down amino acids producing a rise in pH.[11] The pH of the ailing implant in this study was 3.76, indicating that it may have been in the early stages of disease progression and presenting with acute onset of peri-implantitis. A larger sample size could allow for the distinction between acute and chronic presentations of peri-implant disease processes and thus confirm the interpretation of this finding.

The generalizability of this study is limited by the number of subjects enrolled, specifically those with ailing implants. Another limitation is the minimum fluid volume required for paper collection strips and the micro-pH meter to work properly. Not all subjects were able to produce 5 µL of GCF. We found that the amount of fluid collected was dependent on the location of the implant or tooth in the mouth. Anterior sites were much harder to collect fluid from than posterior sites. This could be due to several factors, including airflow into the mouth, gingival biotype, and/or xerostomia. Even with increased proficiency in the fluid collection, obtaining fluid samples from anterior sites remained challenging due to the limited amount of GCF available.

Overall, the goals of this study were achieved. In the majority of cases, it was possible to use gingival fluid collection strips to retrieve enough GCF from the sulcus of implants to measure pH. Additionally, despite the limited sample size of ailing implants,

we observed a difference in pH measured in comparison to healthy implants. If the results from more subjects correspond with the current results, the method of GCF collection and pH measurement as described in this study may be used to early diagnose implant health and disease.

## CHAPTER 5: Conclusions

Early detection of peri-implant disease is of great value to dentists and patients as it can guide treatment and intervention before a questionable implant is deemed a failure. Because probing depths can be technique sensitive among providers with different experience levels, a diagnostic physiological marker that is objective and measurable such as pH, would aid clinicians in the diagnosis of peri-implant disease.[12,13] This study has shown that fluid isolated from paper collection strips in the majority of patients can be used to measure pH of GCF and PICF from natural teeth and dental implants. It is a relatively quick and inexpensive process that could be performed in the dental office. Early detection of disease would greatly benefit the patients by providing them with an ideal treatment at the right time. For the military health system, early detection of disease would also save the DoD the cost of more extensive, time consuming, and expensive care that a failed implant requires. Despite the limited sample size, there was a decreased pH found in the GCF around ailing implants. The pH values of healthy implants and natural teeth were similar. Subjects will continue to be enrolled in the study.

**Table 1.** pH and periodontal status of implants and contralateral teeth.

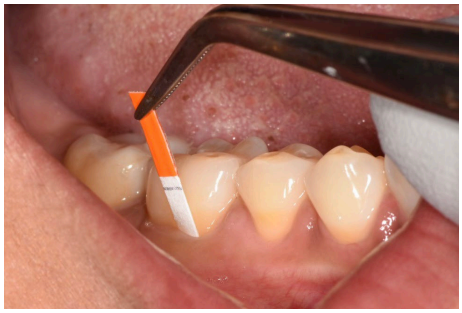
| Subject | Periodontal Status of Implant | pH of Implant | Periodontal Status of Contralateral Tooth | pH of Contralateral Tooth |
|---|---|---|---|---|
| 1 | Ailing | 3.76 | Healthy | 6.21 |
| 2 | Healthy | 5.23 | Healthy | 5.99 |
| 3 | Healthy | 6.85 | Healthy | 6.65 |
| 4 | Healthy | 5.40 | Healthy | 5.46 |
| 5 | Healthy | 5.72 | Healthy | 5.54 |
| 6 | Healthy | 6.68 | Healthy | 5.60 |
| 7 | Healthy | 6.04 | Healthy | 5.99 |
| 8 | Healthy | 6.59 | Healthy | 9.42 |
| 9 | Healthy | - | Healthy | 5.58 |
| 10 | Healthy | 7.16 | Healthy | 7.19 |
| 11 | Healthy | 6.65 | Healthy | 7.07 |
| 12 | Healthy | 6.25 | Healthy | 7.08 |
| 13 | Healthy | - | Healthy | - |
| 14 | Healthy | 6.69 | Healthy | 7.26 |
| 15 | Ailing | - | Healthy | - |

(-) indicates not enough fluid could be extracted for pH sampling

**(A)**


**(B)**

**(C)**



**(D)**

**(E)**


**(F)**

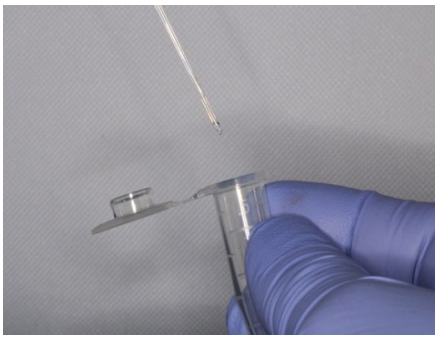


**Figure 1. Fluid collection protocol. (A)** Periopaper Gingival Fluid Collection Strips in sterile packaging. **(B)** One sheet of collection strips used per subject. **(C)** Using cotton pliers, one strip is placed into the sulcus of the collection site. **(D)** Collection strips placed in custom micro-tubes in preparation for centrifugation. **(E)** The two samples from each subject were centrifuged at 15,000 RPM and 4 degrees Celsius for two rounds of ten minutes. **(F)** After centrifugation, isolated fluid was used to measure pH using a micro-pH meter

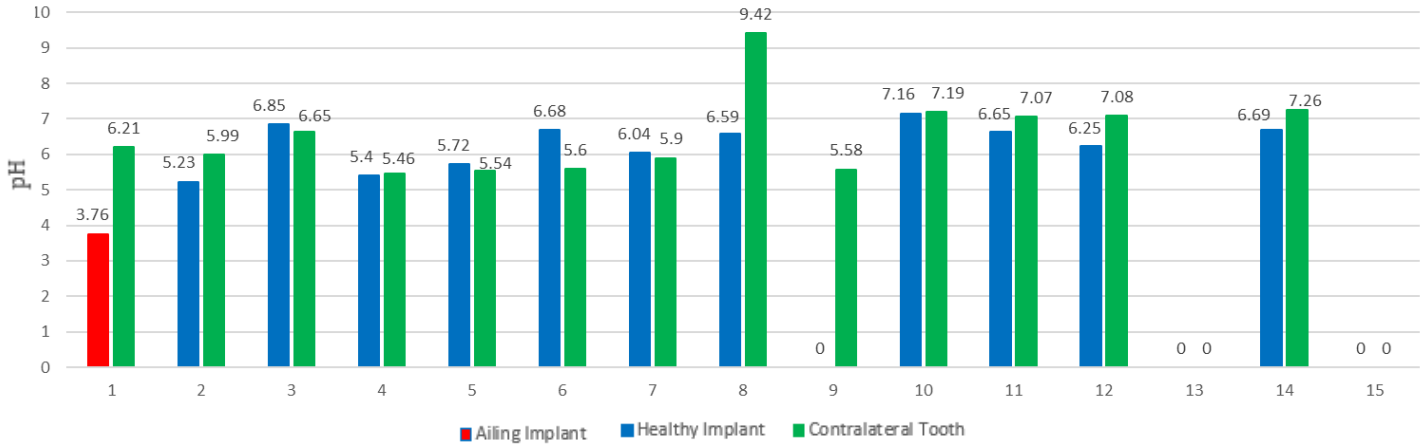



**Figure 2. Graphical representation of results**. The ailing implant is highlighted in red. Healthy implants are highlighted in blue. Control teeth are highlighted in green.

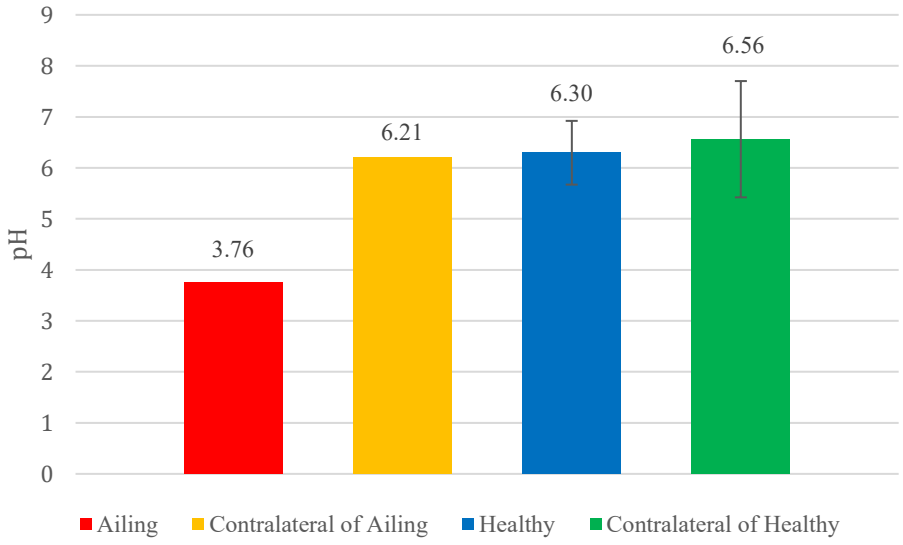



**Figure 3.** Measurable pH of ailing implant (n=1) versus healthy implants (n=1) and contralateral natural teeth (n=11). The pH of the ailing implant site has a much lower pH as compared to healthy implant sites and contralateral natural teeth.

# REFERENCES


1. Caton JG, Armitage G, Berglundh, T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. *Journal of Periodontology*. 2018;89(S1):1820-1834.

2. Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. *Journal of Clinical Periodontology*. 2018;45:S278-S285.

3. Mombelli A, Lang NP. The diagnosis and treatment of peri-implantitis. Periodontol 2000. 1998 Jun;17:63-76

4. Ramanauskaite A, Juodzbalys G. Diagnostic Principles of Peri-Implantitis: a Systematic Review and Guidelines for Peri-Implantitis Diagnosis Proposal. J Oral Maxillofac Res. 2016 Sep 9;7(3):e8.

5. Coli P, Sennerby L. Is Peri-Implant Probing Causing Over-Diagnosis and Over-Treatment of Dental Implants? J Clin Med. 2019 Jul 29;8(8):1123.

6. Nyako E, Watson C, Preston A. Determination of the pH of peri-implant crevicular fluid in successful and failing dental implant sites: a pilot study. Archives of Oral Biology 2005;50(12):1055-59.

7. Faot F, Nascimento GG, Bielemann AM, Campão TD, Leite FR, Quirynen M. Can peri-implant crevicular fluid assist in the diagnosis of peri-implantitis? A systematic review and meta-analysis. J Periodontol. 2015 May;86(5):631-45.

8. Bickel, M. and Cimasoni, G. (1985), The pH of human crevicular fluid measured by a new microanalytical technique. Journal of Periodontal Research, 20: 35-40.

9. Karpavicius, D, Stasikelyte, M, Baseviciene, N, Sakalauskaite, U, Ratkute, S, Razukevicius, D. The determination of pH of peri-implant crevicular fluid around one-piece and two-piece dental implants: A pilot study. *Clin Exp Dent Res*. 2019; 5: 236– 242.

10. Takahashi N. Microbial ecosystem in the oral cavity: metabolic diversity in an ecological niche and its relationship with oral diseases. *Int Congr Ser.* 2005;1284:103–112.

11. Barros SP, Williams R, Offenbacher S, Morelli T. Gingival crevicular fluid as a source of biomarkers for periodontitis. Periodontol 2000. 2016 Feb;70(1):53-64.

12. Badersten A, Nilvéus R, Egelberg J. Reproducibility of probing attachment level measurements. J Clin Periodontol. 1984 Aug;11(7):475-85.

13. Seabra RC, Costa FO, Costa JE, Van Dyke T, Soares RV. Impact of clinical experience on the accuracy of probing depth measurements. Quintessence Int. 2008 Jul-Aug;39(7):559-65