CLINICAL AND MICROBIOLOGICAL EVALUATION OF INTERPROXIMAL OPEN CONTACTS AND PERIODONTAL DISEASE: A CROSS-SECTIONAL STUDY

A Thesis

by

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ABSTRACT

Purpose:

Open contacts causing food impaction are thought to be a contributing factor to localized periodontal destruction. The extent that interproximal open contacts and the dimensions of the open contact contribute to periodontal destruction has not been quantitatively assessed. The purpose of this clinical study is to quantify the relationship between the presence of an interproximal open contact, the dimension of the interproximal open contact to periodontal attachment loss and associated subgingival microbiome.

Materials and Methods:

Twenty-five patients with active periodontal disease with at least one open contact (test) and a contralateral/adjacent closed contact (control) were evaluated by one examiner. The open contact width (mm) was measured using a thickness gauge and subgingival bacterial sampling was performed. The subgingival samples were tested against a 10 species periodontal panel via quantitative polymerase chain reaction (qPCR) by a blinded outside lab. Periodontal parameters including probing depth (PD), recession (REC), clinical attachment loss (CAL), bleeding on probing (BOP), plaque index (PI), and patient-reported food impaction (FI) were recorded. Spearman correlations between open contact dimension and periodontal parameters were analyzed, while differences were examined using Mann-Whitney U, McNemar test and Wilcoxon signed-rank test. Bonferroni corrections were used to control for Type I errors when evaluating periodontal pathogens.

Results:

The median width of the open contact assessed with the thickness gauge was 0.53 mm. The open versus closed sites differed significantly with regard to BOP, CAL, and PD ($p \leq 0.001$, $p \leq 0.001$ and $p=0.038$, respectively). PD and CAL were increased in the open contact site compared to the closed control site ($p \leq 0.001$). Increased width of the open contact was associated with increased PD and CAL ($p=0.003$, $p<0.001$). FI was not related to open contact width ($p=0.335$). Significant differences were found in the amount of *P. gingivalis* ($p=0.004$) and *C. rectus* ($p=0.003$) in test vs control sites. Marginal increases were noted in the amount of *T. forsythia*, *T. denticola* and *Peptostreptococcus micros* ($0.0045<p<0.05$).

Conclusions:

Findings of the current study are consistent with previous studies that demonstrated interproximal open contacts are an important factor in periodontal disease. This study demonstrates quantitatively for the first time that the dimension of the interproximal open contact is directly related to parameters of periodontal destruction. Furthermore, the pathogenicity of the subgingival bacterial profile is directly associated with the presence interproximal open contacts. The findings of this study demonstrate the impact of interproximal open contacts in periodontal disease showing the importance of detection and management of open contacts in clinical practice.

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NOMENCLATURE

BOP	Bleeding on probing
CAL	Clinical attachment loss
CEJ	Cementoenamel junction
Mm	Millimeters
PD	Probing depth
PI	Plaque index
REC	Recession

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Periodontal Disease

Periodontal disease is an important health concern in the United States . In a national study from 2009-2014, an estimated 42% of United States adults 30 years or older with teeth have periodontitis, with 7.8% having severe periodontitis. This study displayed that periodontitis is highly prevalent in the United States adult population [1,2]. This inflammatory disease affects tooth-supporting tissues and exhibits a wide range of microbiological, clinical and immunological manifestations. It is multifaceted in origin, caused by the interactions of infectious agents, host immune responses, genetic susceptibility factors, and environmental exposures [3].

Periodontal disease is categorized by the host mediated response to microbial challenges [4]. *Page and Schroder* described the pathogenesis of inflammatory periodontal disease in four different phases from periodontal health to advanced periodontitis: initial, early, established and advanced lesions. The initial lesion begins as gingivitis, defined as inflammation around teeth without bone loss. At sites of gingivitis, there is an increase of polymorphonuclear leukocytes before becoming an early lesion with increased features and loss of collagen. Next the lesion is established when plasma cells become the main immune cell and persistent signs of acute infection are evident. Lastly, the advanced lesion establishes the shift to periodontitis with the periodontal pocket formation and destruction of alveolar bone and periodontal ligament [3].

The rate at which the progression of periodontal disease occurs has been considered by many authors. *Lindhe et al.* reported the attachment level changes in a Swedish and American

population were slow and continuous, and the majority of sites did not show an increase of attachment loss over time despite having no treatment. They also determined that sites with an advanced attachment loss are more likely to have progression [5]. *Socransky et al.* presented the random burst theory of disease progression, explaining that data was inconsistent with the previous slow continuous model. The random burst model was described as having periods of exacerbation and remission of a disease throughout a patient's life [6]. These findings coalesce in *Jeffcoat's* study in 1991, which found that 76% of sites lost attachment according to the continuous model, while a small subset lost attachment in bursts of activity and remission [7].

Ways to classify periodontal disease have evolved throughout the years [89,10]. Chronic and acute forms of periodontitis exist. Active periodontal disease is defined as interdental clinical attachment loss detectable at ≥ 2 non-adjacent teeth or buccal clinical attachment loss of ≥ 3mm with pocketing of > 3mm detectable at ≥ 2 teeth [4].

Antibiotics are used commonly to treat acute infections, in the initial treatment of periodontal disease, and after periodontal surgery [11–13, 14]. In a systematic review, it was concluded that the use of adjunct antibiotics in scaling and root planing and in surgery resulted in a greater improvement of attachment loss [15]. The antibiotic of choice to treat acute periodontal abscesses is amoxicillin according to the 2004 American Association of Periodontology Position paper [15].

Bleeding on probing has been correlated with histopathology and microbiological changes in periodontal diseases [16,17]. Bleeding on probing is associated with a higher percentage of cell-rich and collagen-poor connective tissue and increase of plasma cells and interstitial space [18,19,20]. These findings led to bleeding on probing being used as a sign of inflammation with other clinical findings [20]. *Lang et al.* found that probing depths over 5 mm had a significantly higher

incidence of bleeding on probing. His data also showed that patients with 16% or more bleeding on probing had a higher chance of attachment loss. *Lang et al.* concluded that bleeding on probing is a limited but useful clinical predictor for disease activity [20]. Conversely, the continued absence of bleeding on probing is a reliable indicator for periodontal health [21]. Due to bleeding on probing being a common clinical test, the pressure at which the probe is applied is of importance. Applying uncontrolled and too light or too hard pressure may result in false negative or false positive readings. If probing over 0.25 N is applied, there is a strong possibility that healthy clinical tissues will bleed, so 0.25 N is the greatest force of pressure that should be applied [20].

The landmark study "Experimental Gingivitis in Man" associated bacterial plaque with gingivitis. The study showed that bacterial plaque is essential in producing gingival changes by demonstrating that gingivitis develops once oral hygiene has been removed [22].

Local factors such as retention of calculus, plaque, overhanging restorations, and open margins hinder the removal of plaque [23]. The accumulation of plaque and hindrance of removal causes maturation of the plaque, presence of certain bacteria, inflammation, destruction, and finally loss of attachment. *Genco & Borgnakke* have reported the presence of local etiological factors including bacteria, specifically *P. gingivalis* and *T. forsythia,* is associated with periodontal destruction [24].

Interproximal Open Contacts

The interproximal contact area is the area of a tooth that is in close association, connection, or contact with an adjacent tooth in the same arch [25]. Food impaction is defined as

the forceful wedging of food into the interproximal space by chewing pressure (vertical impaction) or the forcing of food interproximally by tongue or cheek pressure [26]. There are conflicting views on the subject of interproximal open contact literature and its effect on food impaction. The role of interproximal open contacts and periodontal destruction has not been clearly established [27].

Kepic & O'Leary found no difference in periodontal breakdown in open versus closed contact sites as long as oral hygiene status was maintained [28]. Another study by *O'Leary et al.* evaluated periodontally healthy male dental students and the relationship with proximal contact and marginal ridges classified by their orthodontic status. Interestingly, the study excluded students with alveolar bone loss and did not report a relationship between interproximal open contacts and increased probing depth or destruction [29].

When *Larato et al. 1971* evaluated 206 infrabony lesions in 121 human skulls, only 18% were found to be associated with factors able to cause food impaction. He found a large number of infrabony lesions not related to food impaction, suggesting that food impaction does not have a major influence on the pathogenesis of interproximal infrabony lesions. However, there were many limitations on the study, mainly arising from the fact that it was performed on skulls. None of these dental skulls had dentals restorations and the dental history of these subjects was unknown [30].

Geiger et al. 1974 investigated the relationship between periodontal disease and spacing by studying patients at a dental school. He found among the 516 individuals, 11% of interproximal contacts had some spacing. The spacing was most common in the maxillary incisors and cuspids, followed by the mandibular anteriors. He found the incidence and severity of spacing showed bilateral symmetry. The results showed that in the full dentition as the spacing

increased the periodontal destruction increased, except in cases where there was spacing in more than 40% of the dentition. It was suggested that if a large arch is present and a great amount of spacing is present, then the dentition is not conducive to periodontal destruction. When observing an open contact with a contralateral closed contact, teeth with spacing did not have an increased amount of destruction compared to those without spacing in the contralateral site. In the discussion of open contacts influence on periodontal disease, *Geiger et al*. state that there is a slight association with periodontal destruction and spacing for a full dentition with a stronger association in the anterior segments. However, when specifically comparing the open and closed contacts on different sides, there was no relationship found between probing depth and open contacts. This suggested the association could then be due to a masking effect of averaging scores and that there was no true significance. *Geiger et al*. state that these conflicting results support the notion that the presence of spacing cannot be predictably linked to periodontal disease [31].

Proximal open contacts have been suggested to be one of the etiological factors associated with food impaction and a modifying factor in periodontal disease. The lack of the contact has been considered to be similar to food impaction, poor margins, and calculus deposits as a secondary etiological agent in periodontal disease [32]. *Prichard* suggested that food impaction was a primary extrinsic factor to the pathogenesis of vertical bone defects [33,34].

In a landmark study, *Hancock et al*. evaluated a group of 40 healthy young adult naval recruits who had a full dentition and no dental treatment, other than the dental examination performed at commencing active duty and confirmation they had no systemic diseases. Due to these inclusion criteria, *Hancock et al*. mentioned this population could be considered as having the highest level of oral health among the naval recruits. He found gingival inflammation,

considered to be moderate or severe, in 80% of areas which were examined but he did not find a significant relationship between gingival index or the pocket depth and open contacts. Food impaction was determined by a presence of food wedged interproximally. Four percent of the 1040 areas exhibited food impaction. Pocket depth was lowest in areas with a tight contact and higher in areas of loose and open contacts. Food impaction in open contacts was found to be related to increased pocket depth, leading to the statement that food impaction contributes to periodontal disease. Thus, recommendations henceforth were to remove interproximal plaque accumulation and ensure open contacts are not impacting food. Open contacts by themselves without food impaction were not found to be related to an increased pocket depth [35].

The relationship between proximal contacts and periodontal disease was studied by *Jernberg et al.* in a cross-sectional split mouth study. One hundred and four subjects with an open contact and contralateral closed contact were assessed interproximally. The population was almost equally divided male and female with the mean age of 42.8 and range from 21 to 80 years old. The inclusion criteria did not take periodontal status into consideration, but the exclusion criteria removed subjects who had undergone scaling and root planing in the last 4 months or had a history of periodontal surgery. GI, BOP, PD, CAL, calculus, and food impaction were recorded. The width of the open contact was measured by using 0.1mm metal gauges. Periodontal parameters were compared between an open contact and a contralateral closed contact. Anterior contacts were 75% of the open contacts and food impaction was determined by visual inspection of fibrous food or patient reported food impaction. A difference of clinical attachment loss of 0.48 mm was found at the open vs contralateral closed contact showing a significantly greater probing depth and attachment loss at the open contact site. A small but statistically significant relationship was found between food impaction and probing depth

between closed and contralateral open contact sites. *Jernberg et al.* mentioned that a closure of the open contact may be suggested to alleviate food impaction [36].

Koral et al. 1981 presented evidence that the influence open contacts had on bone destruction may be dependent on the periodontal status of the patient. In only early periodontitis patients, as classified by the 1986 American Dental Association, open contacts had a statistically significant reduction of 2.4% bone height compared to the contralateral site [37]. No association was found between restored and not restored contacts in terms of bone loss. Open contacts in gingivitis, moderate periodontitis, and severe periodontitis were not found to have increased bone loss [38].

Reports in the literature provide conflicting views on the effect of open contacts in the periodontium. Although the role of shape of contour of proximal tooth surfaces on the healing of gingival tissue is a known influence due to the notion that the healing gingiva does not necessarily follow the contour of underlying bone [39,40]. It has never been shown that the size of the open contact is related to the effect of food impaction and periodontal destruction, or whether the extent of open contacts play a role in this destruction. As mentioned previously, *Jernberg et al.* did not find a relationship between the width of open contact and periodontal destruction [36].

Implant Interproximal Open Contacts

Interproximal open contacts between implant supported restorations and natural teeth are a multifactorial implant complication [41]. There are differing reports in the literature of the effect of open contacts on marginal bone destruction. A recent retrospective study found 34.1% of mesial contacts were open after 10 years with 48% of mesial contacts being open or loose. The

study did not find an association between interproximal open contacts and peri-implant inflammation, with an exception of the distolingual implant surface. This study noted patients were more aware of the food impaction around the implant crown [42]. In a separate study *Saber et al.* found a similar incidence of interproximal contact loss between implants and adjacent natural teeth of 32.8%. This article found sites with an open contact were 2.24 times more likely to present with bleeding on probing, and marginal bone loss was statistically significant. The study concluded that there was a positive relationship between interproximal open contact and marginal bone loss in implants [43].

Latimer et al. 2021 performed a cross-sectional study on 142 implants adjacent to a natural tooth in which 54.2% of implants were found to have interproximal open contacts. The implants which had an open interproximal contact were found to be highly associated with peri-implant mucositis and peri-implantitis. Higher probing depths, plaque indexes, and gingival indexes were also found at sites with open contacts. The mesial surface of the open contact was open 68.5% of the time. *Latimer et al.* concluded that interproximal open contacts between implants and natural teeth are indicators for increased PD and peri-implant disease [44].

Bacteria

Holt et al. 1988 showed induction of periodontitis upon oral implantation of red complex bacteria into non-human primates [45]. *Socransky* defined bacterial communities in subgingival plaque using data from plaque samples. He found that there were 5 major complexes. The first complex consisted of *T. forsythia, P. gingivalis* and *T. denticola,* which were labeled as the red complex due to their strong association with bleeding on probing. The red complex was

categorized together based on the association with severe forms of periodontal disease. These bacteria exhibit a strong association with increased pocket depth. When *P. gingivalis* was found alone or in combination with the other 2 red complex species, there exhibited a deepest mean pocket depth. Sites in which none of the species were found proved to have the shallowest mean pocket depth while in sites where all 3 red complex species were found, there proved to have the deepest pocket depth [46].

P. gingivalis is an anaerobic bacterium implicated in periodontal disease and other inflammatory systemic conditions [46,47]. At very low levels, it triggers changes in the composition of oral microbiota leading to inflammatory bone loss, enabling this low abundance species to disrupt host-microbial homeostasis and cause inflammatory disease [48]. *P. gingivalis* has evolved strategies to evade host immune systems and impairs the innate immune response, causing disruptive changes in the microbiota. *P. gingivalis* has a capsule and fimbriae which work together to increase its virulence. The capsule increases resistance to phagocytosis and decreases chemotaxis of neutrophils. The bacterial fimbriae are important in motility and chemotaxis. Proteinases produced by *P. gingivalis* include hydrolytic, proteolytic, and lipolytic *enzymes* [49]. Gingipains are arginine-specific cysteine proteinases of *P. gingivalis* which exhibit C5 convertase-like activity. This causes an enhancement of inflammation while impaired killing capacity for leukocytes. *P. gingivalis* also secretes serine phosphatase which inhibits the synthesis of interlukin-8 by epithelial cells, causing a delay in neutrophil recruitment. The impaired leukocyte and neutrophil recruitment may allow other species to grow in the biofilm thereby increasing the bacterial count. This uncontrolled growth leads to inflammatory destruction and tissue breakdown. The *keystone-pathogen hypothesis* supports the idea that

certain microbial pathogens, specifically *P. gingivalis,* can cause a homeostatic microbiota to develop into dysbiosis [50].

T. forsythia, previously named *Bacteriodes forsythus*, is a gram-negative anaerobic bacillus which is associated with forms of periodontal disease and infections. Multiple virulence factors have been identified including proteases and secreted proteins. *T. forsythia* is closely related to *F. nucleatum,* which is part of the orange group. *T. forsythia* is rarely seen as alone but instead *F. nucleatum* acts as a bridging bacterium to facilitate *T. forsythia* and other bacteria to colonize [49,51]. *T. denticola*, the final pathogen of the red complex, is a gram-negative anaerobic spirochete which has been established to have a strong relationship with deep probing depths and inflammation. *T. denticola* co-aggregates with *P. gingivalis* and *F. nucleatum.* The production of multiple factors such as collagenase, hyaluronidase and other hydrolytic enzymes contribute to *T. denticola's* virulence [49].

The second complex observed by Socransky was the orange complex which works in close association with the red complex. The orange complex consists of *F. nucleatum, F. periodonticum, P. micros, P. intermedia, P. nigrescens, Streptococcus constellatus, E. nodatum C. showae, C. gracili,s* and *C. rectus.* As previously mentioned *F. nucleatum* is a bridging bacteria whose presence facilitates red complex bacteria [46]. *Ali et al.* found that in subgingival plaque samples, *P. intermedia* was always found in the presence of *F. Nucleatum* [52]. *P. micros* is a gram-positive organism which is recognized as pathogenic in medical infections and is part of the orange complex. *P. micros* is associated with progressive periodontitis and occurs more frequently in active periodontitis lesions than inactive ones [53,54]. *Von Troil-Linden et al.* discovered that *P. intermedia, C. rectus,* and *P. micros* were found at greater concentrations in saliva samples from subjects with advanced periodontitis than non-periodontitis subjects [55]. Long

lasting changes in the subgingival microflora can be established by even a single course of periodontal treatment [56]. *Rams et al.* evaluated *C. rectus* in periodontitis and the response after debridement. The subgingival *C. rectus* was collected via paper points and was recovered from 80% of the 1654 periodontitis patients who were sampled. It was found that there was an inverse relationship between a positive culture of *C. rectus* and increasing age. *C. rectus* was found to be positively correlated with disease progression as many authors have confirmed. Once debridement of a patient occurred the *C. rectus* percentage decreased from 8.2% to 0.7%. *C. rectus* was also found to have high susceptibility to tetracycline hydrochloride, metronidazole, penicillin G and ciprofloxacin [57].

Socransky et al. observed three other complexes, identified as the yellow, purple and green complexes. *Streptococcus mitis*, *streptococcus sanguis,* and *streptococcus oralis* form the yellow complex. The purple complex consists of *Actinomyces odontolyticus* and *Veillonella parvula*. The green complex consists of *E. corrodens*, *Actinobacillus actinomycetemecomitans serotype a*, and the *Capnocytophaga* species. Members of the green and yellow complexes were less commonly observed with bleeding on probing or associating with the orange and red complexes. The purple complex was found to be related to the orange, green and yellow complex to a much lesser extent [46]. It is suggested that some environments may be more selective for one complex and antagonist for other complexes [58].

Actinobacillus actinomycetemcomitans (*Aa*) is a gram-negative, nonmotile, capnophilic coccobacillus which has 10 different serotypes [59]. According to *Socransky et al., Aa* did not cluster with the other species so it was not placed in a colored complex. *Aa* related poorly to the red and orange complexes which suggested that the therapeutic interventions may not be effective to both *Aa* and orange or red complex bacteria [46]. Serotype B is often seen in localized

juvenile periodontitis and its primary niche is in the oral cavity. *Aa* can cause severe infections in the human body including brain abscesses and endocarditis. An increased prevalence of *Aa* and elevated serum antibiotics targeting *Aa* are found in localized juvenile periodontitis patients [59,60]. There is a strong correlation between *Aa* and periodontal pockets. *Aa* invades the gingival connective tissue and creates severe pathogenic products. Host defense mechanisms are inhibited including leukotoxin and PMNs. There is also a destruction of tissue via LPS and collagenases [60,59].

There are many theories as to what role pathogens play in causing inflammatory periodontal disease. There has been a controversy between two points of view, one mentioning a "non-specific theory" while another mentioning a "specific theory." The non-specific theory describes oral bacteria colonizing to form plaque. This plaque triggers inflammation and periodontal breakdown. All the plaque as a whole is thought to cause the destruction regardless of the composition of bacteria. Therefore, plaque control would be necessary to control periodontal breakdown. The specific theory, in comparison, hypothesizes that a single pathogenic species causes inflammation in periodontal destruction [61].

Bacterial Sampling

The technique in which bacteria is sampled is important to the outcome of microbiological assays [62,63]. The bacterial plaque distribution changes in composition during development of gingivitis and periodontitis [22,64]. This has been shown to have a non-homogenous distribution of bacteria by use of ultrastructural observations of bacterial plaque [65]. Antibody techniques to establish bacterial composition do not accurately show the precise distribution of

bacteria [66]. Paper point absorbent sampling was designed to investigate the ability to accurately determine the "whole site" bacteria present. This would include not only the bacteria at the coronal portion of the pocket, which would be easier to culture due to the ease of access and aerobic nature, but also the bacteria at the apical portion of the pocket [67]. *Baker et al.* evaluated the homogeneity of paper point sampling and found that a non-homogenous distribution of bacteria was not adequately characterized by paper point sampling. However, if the bacteria was homogenous it was accurately characterized. He found that a misrepresentation of the samples was likely due to the saturation of the paper point at the coronal portion of the sample site [67]. However it has been pointed out that paper point sampling can categorize diagnostic markers for periodontal disease and may still be useful in microbiological assays [63].

Quantitative Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a technique for quantifying nucleic acid molecules in bacterial samples [68]. The general concept of PCR has not changed since 1985 and has furthered research in areas of biology and technology. Real time PCR, also named quantitative PCR or qPCR, monitors DNA amplification in real time through monitoring fluorescence [69,70]. The amount of fluorescence reflects the amount of DNA in the sample at a specific point in time. In the initial cycles the level of fluorescence is low and not able to be detected, but there reaches a point when the fluorescence is detected and corresponds to the number of DNA strands in the sample. This is called the quantification cycle and allows the quantity of DNA strands to be distinguished according to a calibration curve from standard samples with known concentrations [71,72]. QPCR uses automated equipment to quickly test thousands of samples for biologic activity and quantify DNA without concern of cross contamination.

Aims & Hypotheses

The primary aim of this research was to establish a relationship between the presence of an open contact and loss of periodontal attachment in patients with active periodontitis. An additional primary aim was to establish a relationship between width of an open contact and pocket depth, attachment loss, gingival inflammation, plaque accumulation and food impaction in patients with active periodontitis. The secondary aim was to determine if a relationship exists between subgingival microbiome and an open contact. We hypothesized that open contacts were related to increased periodontal parameters (PD, CAL, BOP, PI) and food impaction. This hypothesis was based on the common clinical finding of an increased probing depth at a site of an open contact in a patient with periodontitis. Additionally we hypothesized that as the width of open contact increased there would be a decrease in periodontal parameters. It is generally rationalized that smaller open contacts entrap food while larger open contacts allow for food to pass through without being impacted. As discussed previously, food impaction has been related to an increase in periodontal parameters [35].

CHAPTER II

MATERIALS AND METHODS

Patient Enrollment

The Institutional Review Board of Texas A&M University of College of Dentistry (TAMUCOD), Dallas, Texas reviewed and approved the protocol for this cross-sectional clinical trial (IRB2019-1201-CD-EXP). A total of 25 patients, 13 males and 12 females, aged 29 to 76 years with mean age of 53 were enrolled in the study from April 2021 to December 2021. No patients dropped out or were removed from the study. Patients were recruited from the Texas A&M College of Dentistry. All patients had active periodontal disease, defined as interdental clinical attachment loss detectable at ≥ 2 non-adjacent teeth or buccal clinical attachment loss of $\geq$ 3mm with pocketing of $>$ 3mm detectable at ≥ 2 teeth [4]. All patients had at least one open contact, defined as a lack of integrity between two adjacent teeth which can be felt through passage of floss with no resistance. Of the open contacts observed, 15 were in the posterior molar and premolar region while 10 of the open contacts observed were from the anterior incisor region. Patients also were required to present with a closed contact between an adjacent or contralateral tooth. A contralateral closed contact would be the contact between the same type of teeth in the arch but on the other side of the mouth. An adjacent closed contact would refer to the contact between two teeth next to the open contact. Radiographs of the teeth in question within the past 12 months were required. Patients were also required to be over the age of 18.

Patients were excluded if they met at least one of the following criteria: active periodontal therapy (surgical or non-surgical) in the previous 6 months, antibiotic treatment in

the previous 6 months, presence of implants in the test and/or control teeth, and interproximal restoration performed < 12 months previously. Patients were also excluded if the cementoenamel junction (CEJ) was unable to be identified due to a restoration or crown involving the CEJ.

Once patients were determined to fit inclusion and exclusion criteria they were asked to participate in the study. Patients asked questions and agreed to participate in the study. Patients read and signed an informed consent and HIPAA authorization forms which detailed the clinical examination and the risks of the study. All interventions were considered to be within the standard of care. Patients were gifted a Visa Gift Card of $30 for their participation in the study.

Clinical Protocol

Clinical examination was performed by one examiner, Dr. Sarah J. Kelly (S.J.K.). A stone model of a patient's dentition with five open contacts was measured by S.J.K. in one session and remeasured again 48 hours later. The same protocol was used to assess intra-examiner calibration for probing depths accomplished by one quadrant probing depth, recession, and bleeding on probing of a patient and repeating the measurements 48 hours later. These measurements were assessed by kappa statistics. The inter-examiner reproducibility assessed by kappa statistics was accepted with an agreement of ≥61% on the dimension of the open contacts. The number 61% was chosen because that value was defined by *Landis & Koch 1977* as the minimum kappa value indicating "substantial" agreement. Kappa statistic agreement of ≥61% was confirmed [73].

The following baseline clinical parameters were evaluated. A UNC-15mm probe was used to measure probing depth, clinical attachment level and recession. The PDs were evaluated

in 6 sites per control and open contact teeth and were determined to be the distance measured from the base of the pocket to the most apical portion of the gingival margin [74]. The presence of bleeding on probing (BOP) was recorded after probing and defined as present or absent. Plaque index (PI) was determined by visual inspection according to *Silness and Löe* and was given the value from 0 to 3 [75]. Recession (REC) was determined by measuring the distance of the gingival margin to the cementoenamel junction (CEJ). Clinical attachment loss (CAL) was calculated by adding the probing depth to the recession. Food impaction was determined by asking the patient (with yes or no responses) if they have noticed food impaction in open contact sites. Dimension of open contact was measured by using a 32 blade Kobalt feeler gauge which measures millimeters to the thousandth decimal place. The 32 blades were between sizes 0.038 mm and 0.889 mm. Different size gauges were fitted into the interproximal open contact until the most snug fit gauge was determined. Multiple size gauges were used if a site was wider than 0.889 mm. Once the gauge was determined, the next larger gauge was attempted and if it failed, determined the correct size of gauge as the previous measurement.

Bacterial sampling was performed via paper points. Approximately 3-5 paper points were placed in the sulcus of the control and open contact tooth for 15 seconds and then placed in sterile tubes not containing a solution. Paper points samples were mailed to Access Genetics OralDNA® lab to be analyzed via qPCR. Per Access Genetics guidelines the samples were overnighted to the lab and were analyzed within the week of being sampled. In order to determine a consistent sample site, the bacterial sampling site was determined to be the deepest probing depth in the open contact site. If two sites had equal probing depth one was chosen arbitrarily. The control bacterial sampling site was chosen by determining the contralateral equivalent of the open contact bacterial sampling site. For example, if the mesiofacial contact of

#7 was sampled as the test site then #10 mesiofacial contact would be sampled as the control site assuming the #9/10 contact is closed. If the contralateral contact was not closed and an adjacent closed contact site was the control it would be sampled instead. Following the example stated previously with the test sample of mesiofacial contact of #7, the mesiofacial contact of #6 or mesiofacial contact of #8 could be sampled as a control site.

Samples were anonymously coded and sent to Access Genetics OralDNA® labs for qPCR bacterial analysis of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, eubacterium nodatum, fusobacterium nucleatum, Prevotella intermedia, campylobacter rectus, Peptostreptococcus micros, Eikenella corrodens,* and *Capnocytophaga species.* These specific oral pathogens were selected by Access Genetics OralDNA® labs as a part of their 11 oral periodontal pathogen test. Results of bacterial sampling were uploaded to an online portal which included all samples quantitative results. OralDNA® labs were blinded to the identity of test or control samples.

CHAPTER III

RESULTS

A total of 25 patients were clinically assessed and bacterial samples evaluated via quantitative polymerase chain reaction. Table 1 presents descriptive statistics for periodontal indices of open and closed contact sites.

The median width (interquartile range) of the open contact assessed with the feeler gauge was 0.53 (0.395, 1.140) mm. Food impaction was reported in 17 out of the 25 patients, while 8 of the 25 patients did not report food impaction. The median plaque index was 2 for the twenty five patients. Group comparisons were analyzed with Wilcoxon signed-rank test for all tests except bleeding on probing and food impaction which were analyzed with McNemar's test. Median probing depth was 5mm at the open contact site and 3.5 mm at the closed contact side. There was an increase in probing depth in the open contact vs the closed contact site ($p<0.001$) (Figure 1). Median clinical attachment level at the open contact sites was 5 mm while it was 3 mm at the closed contact sites. There was an increase in clinical attachment loss at open versus closed contact sites ($p<0.001$) (Figure 2). Bleeding on probing was also found to be significantly different at the open contact versus closed contact site ($p=0.038$). The open contact sites had 92% bleeding on probing while the closed contact sites presented with 74% bleeding on probing (Figure 3).

Spearman correlations between open contact dimension and periodontal parameters were analyzed, while differences were analyzed using Mann-Whitney U and Wilcoxon signed-rank test. Table 2 depicts the correlation of PD, CAL, and BOP to the presence of food impaction and the plaque index. PD, CAL and BOP were not found to be statically related to presence of food impaction or plaque index. Table 3 depicts the width of open contact's effect on PD, CAL, BOP,

and PI. Bleeding on probing and plaque index were found to be not significantly correlated to the open contact width ($p>0.719$, $p>0.27$). Probing depth and clinical attachment level were found to be highly significantly correlated to the open contact width ($p=0.003$, $p<0.001$). As the width of the open contact increased there was an increase in PD and CAL (Figure 4, Figure 5).

Interestingly, the patient-reported food impaction was not related to open contact width ($p=0.335$) (Figure 6). The plaque index was found not to be significant in regard to feeler gauge width ($p=0.248$) (Figure 7). The probing pocket depth and clinical attachment level in the open contact sites did not differ significantly between sites with and without food impaction ($p=0.170$, $p=0.176$) (Figure 8, Figure 9).

Wilcoxon Signed Ranks Test was used to evaluate the differences in bacterial samples in the open and closed contact sites. Bonferroni corrections were used to minimize Type I errors when evaluating periodontal pathogens. The p-value of 0.05 was divided by 11 to make the corrected p-value 0.0045. Eleven was chosen due to the 11 periodontal pathogens being analyzed. P-values below 0.0045 were deemed highly significant. P-values between 0.05 and 0.0045 were deemed marginally significant. Figure 10 depicts the bacterial data at open and closed contact sites for all pathogens. Significant differences were found in the amount of *P. gingivalis* ($p=0.004$) and *C. rectus* ($p=0.003$) in test sites compared to the control sites (Figure 11, Figure 12). Marginal statistically significant increases were noted in the amount of *T. denticola, T. Forsythia* and *P. micros* ($0.0045<p<0.05$) (Figure 13). There was found to be no statistical significance in the amount of *Aggregatibacter actinomycetemcomitans, Eubacterium nodatum, Fusobacterium nucleatum, Prevotella intermedia, campylobacter rectus, Eikenella corrodens,* or *Capnocytophaga species. Prevotella intermedia*'s difference in quantity at open versus closed contacts was at the level of $p=0.051$ while *Fusobacterium nucleatum* was at the level of $p=0.058$.

Both values of *P. Intermedia* and *F. Nucleatum* were narrowly beyond marginal significance. *A. actinomycetemcomitans* was only detected in a single patient. The data from the samples in this study (n=50) in comparison to the concentration of pathogens in all samples from Access Genetics Oral DNA (n=47,169) is shown in Figure 14.

CHAPTER IV

DISCUSSION

This cross-sectional, single-center, clinical study demonstrates that an interproximal open contact in patients with periodontal disease is a local factor for increased periodontal destruction. This study confirms that there is a strong direct association between interproximal open contacts and increasing periodontal parameters of PD, CAL, and BOP in patients with periodontal disease. This study provides evidence that both the presence and dimension of an open contact are directly associated to the severity of periodontal destruction.

Our study supports *Jernberg's* findings that a site with an open contact has greater destruction than the contralateral closed contact site [36]. We found an increase in probing depth, clinical attachment level, and bleeding on probing in an open contact site compared to a closed contact site by highly significant levels. Conversely *Geiger*, *Larato* and *O'Leary* each had previously stated that open contacts do not have a significant effect on the amount of destruction of the periodontium.[29–31] *Hancock* agreed with these findings that pocket depth was not related to the contact type. Our differing findings could be due to our difference in cohort of patients. [35]*O'Leary* observed dental students, *Larato* had been observing human skulls, and *Geiger* was observing any patient at the dental school. *Jernberg* rejected patients if they had previous periodontal surgery but had no inclusion or exclusion criteria based on their periodontal status. In this study we specifically studied only patients who had periodontal disease and how open contacts affect those individuals. For this reason, we can understand why our data could contradict previous studies where a small number of patients had periodontal disease. This is the first study to have

specifically looked at open contacts in patients with periodontal disease and found there to be a high significance in the amount of destruction at a site of open contact versus a closed contact site.

This is also the first study to find a correlation between width of open contact and periodontal parameters. *Jernberg et al. 1983* found no association between the width of open contact and periodontal parameters but stated "trends toward greater attachment loss and lower debris index with larger size of open contact are observed" [36]. That trend was confirmed in our study. As the width of open contact increased in millimeters the clinical attachment level and probing pocket depth also increased. This finding goes against common assumptions and the researchers' hypothesis. Researchers hypothesized that a small open contact is most damaging to the periodontium and as an open contact increases in width it becomes more cleansable with less food impaction and lower probing depths. Interestingly in this study the food impaction was not related to contact width and the smaller width open contacts had less destruction while the larger width open contact width had a greater amount of destruction. These findings go against common beliefs and demonstrate that not only the presence of an open contact but a larger width of contact is related to an increase in periodontal parameters.

The landmark study by *Hancock* found that food impaction was related to an interproximal open contact and that increased probing depths was related to food impaction. Our findings find that food impaction was not related to an increase in PD, CAL or contact width. There are a few differences between the various studies which may explain the differences in results. In *Hancock'*s study, he determined food impaction by a visual presence of food, while our study used patient reported food impaction. *Hancock's* study's cohort were healthy naval recruits while our study specifically looked at patients with active periodontal disease which is likely to be the main reason for the differing results. Interestingly only 4% of *Hancock's* naval recruits had food impaction

while 68% of patients in our study reported food impaction. This demonstrates that food impaction certainly is not unrelated to interproximal contacts but instead likely a part of the multifactorial etiology and a local factor as well. Both studies could agree that food impaction is related to open contacts. Our study found that food impaction was not related to an increase of probing depth, clinical attachment loss or width of open contact, but considering 68% of our subjects reported food impaction it still may be a factor which should be taken into consideration.

Additionally, open contacts demonstrated increased levels of periodontal pathogens specifically *P. gingivalis* and *C. rectus* in comparison to the closed contacts. *P. gingivalis* and *C. rectus* are known to be implicated in periodontal disease and are commonly found in areas with periodontal destruction, however these pathogens have not been previously associated with open contacts until now. *T. forsythia, T. denticola,* and *P. micros* were deemed to present in marginally significant amounts. These red and orange complex pathogens are frequently associated with periodontal disease and increased probing depths as was found in the open contact sites. *Rams et al*. found both *C. rectu*s and *P. micros* to be associated with progressing periodontitis and many authors have discussed the relationship between the red complex bacteria P. *gingivalis, T. forsythia* and *T. denticola* and increased probing depths and periodontal disease [46,53,57].

Previous literature mentions the association these bacteria have with periodontal disease but not an association with interproximal open contacts. The patients in our study had been diagnosed with periodontal disease so while an increase in these bacteria is to be expected, the presence of these bacteria significantly in open contacts versus a closed contact in the same patient is not. This leads us to wonder, has the microbiome shifted due to the presence of an open contact? Or instead has the open contact influenced the periodontal breakdown leading to an increase in probing depth? Did this periodontal breakdown then by nature cause a microbiome shift? We

hypothesize it is likely the latter, that the increased periodontal breakdown happens prior to the bacterial shift but further studies will need to confirm this hypothesis.

CHAPTER V

CONCLUSION

The findings of the current study are consistent with previous studies that demonstrate interproximal open contacts are an important local factor in periodontal disease. This study demonstrates quantitatively for the first time that the dimension of the interproximal open contact is directly related to parameters of periodontal destruction. Furthermore, the pathogenicity of the bacterial profile of the subgingival microbiome is directly related to the presence of an interproximal open contact. The findings of this study quantitatively highlight the impact of the interproximal open contact in patients with periodontal disease underscoring the importance of detection and management of this local factor in clinical practice.

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APPENDIX

FIGURES

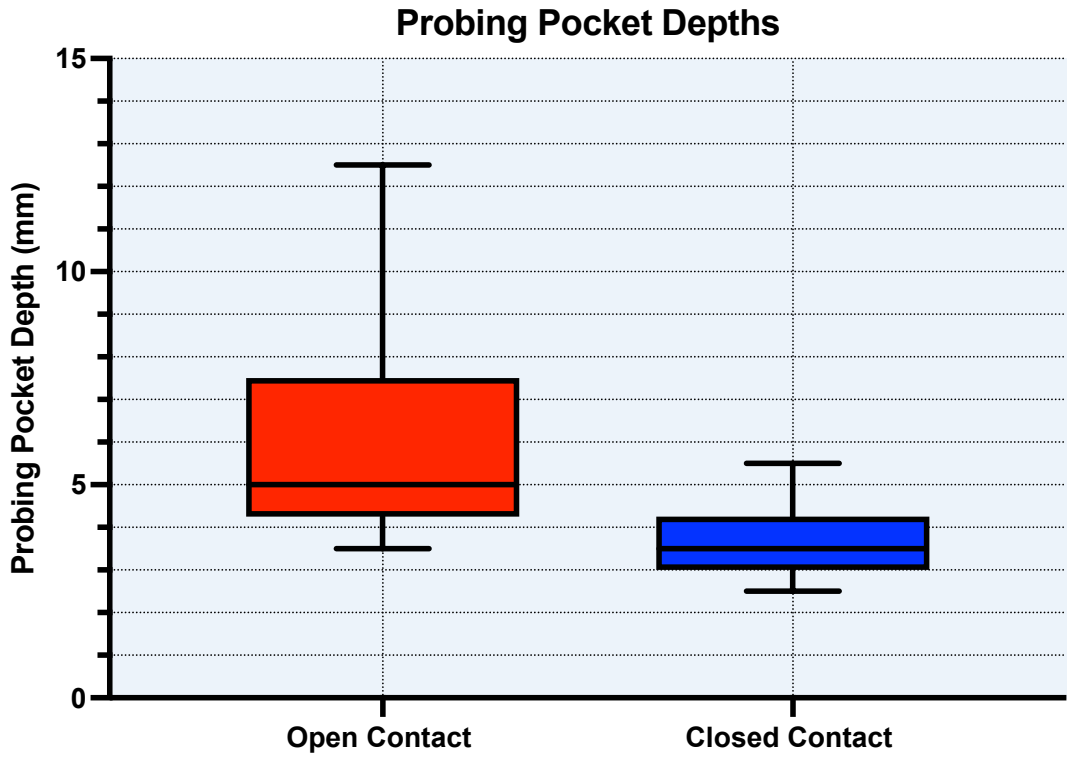

Figure 1: Probing pocket depth difference in open and closed contact sites

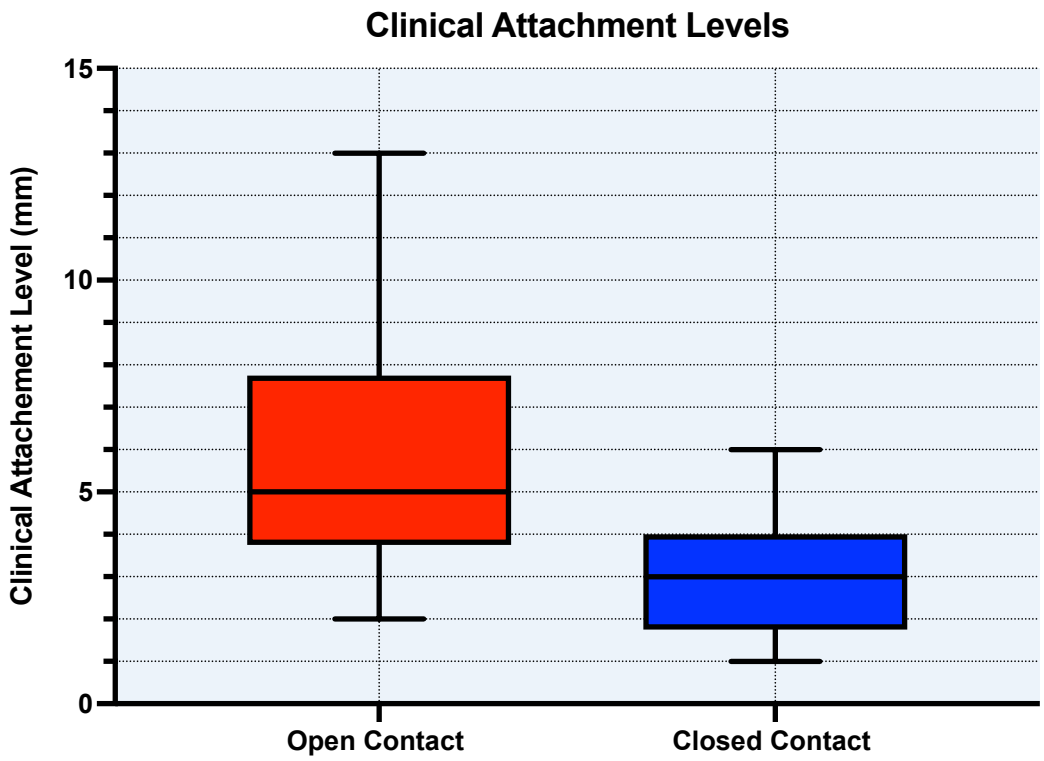

Figure 2: Clinical attachment level difference in open and closed contact sites

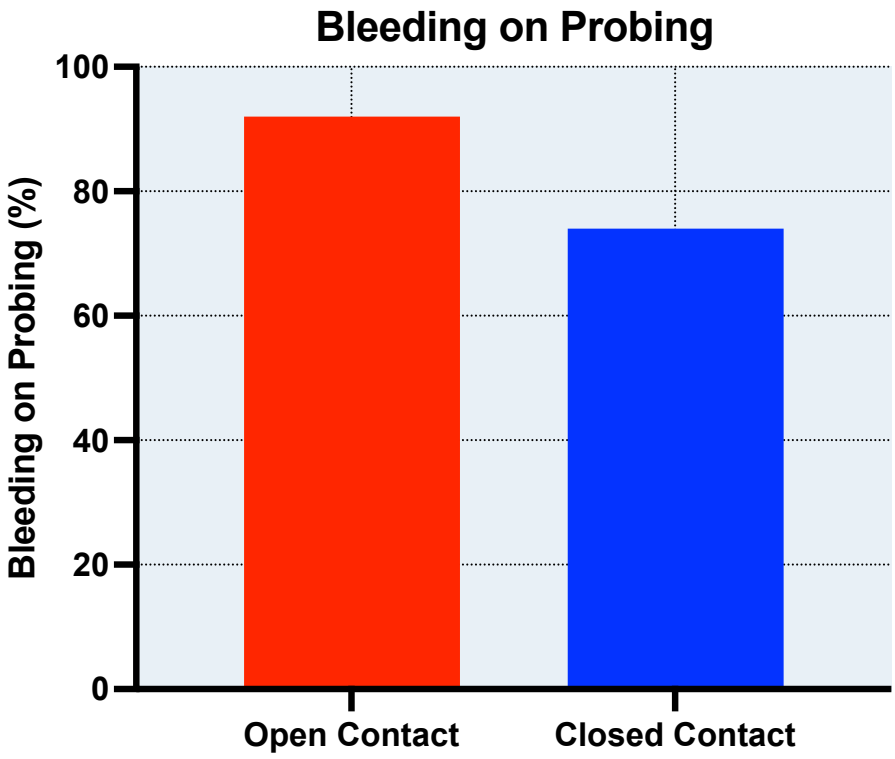

Figure 3: Bleeding on probing difference in open and closed contact site

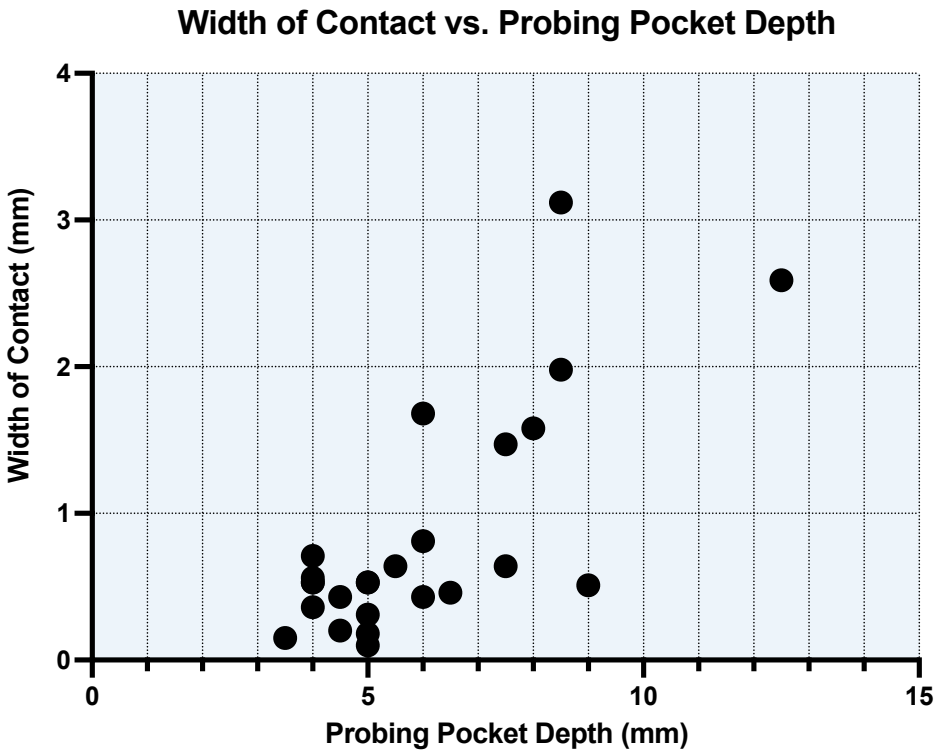

Figure 4: Width of open contact vs. probing pocket depth

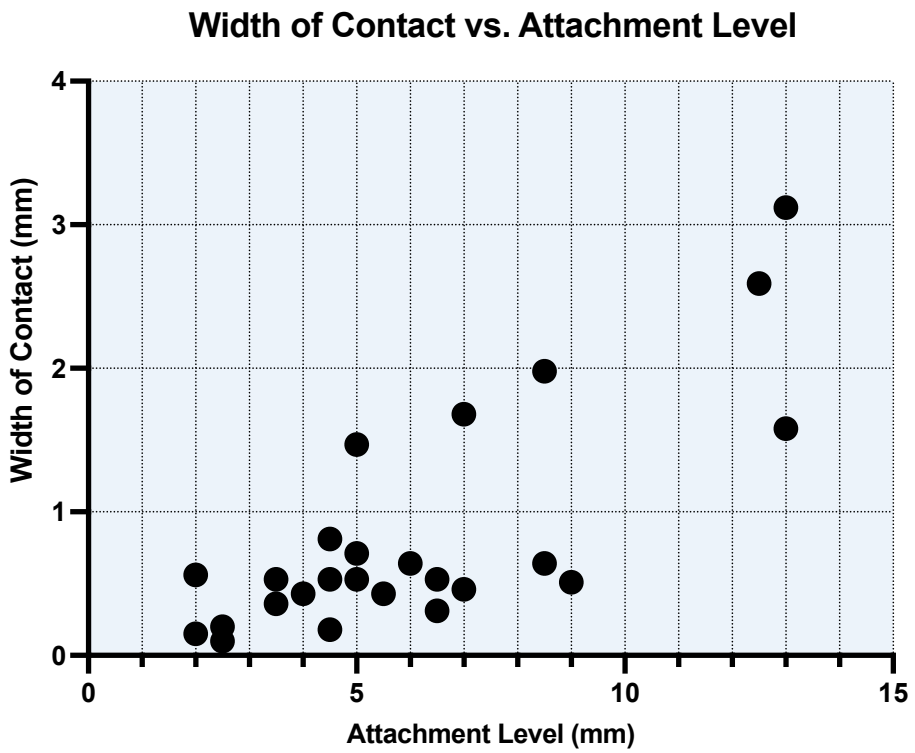

Figure 5: Width of open contact vs. clinical attachment level

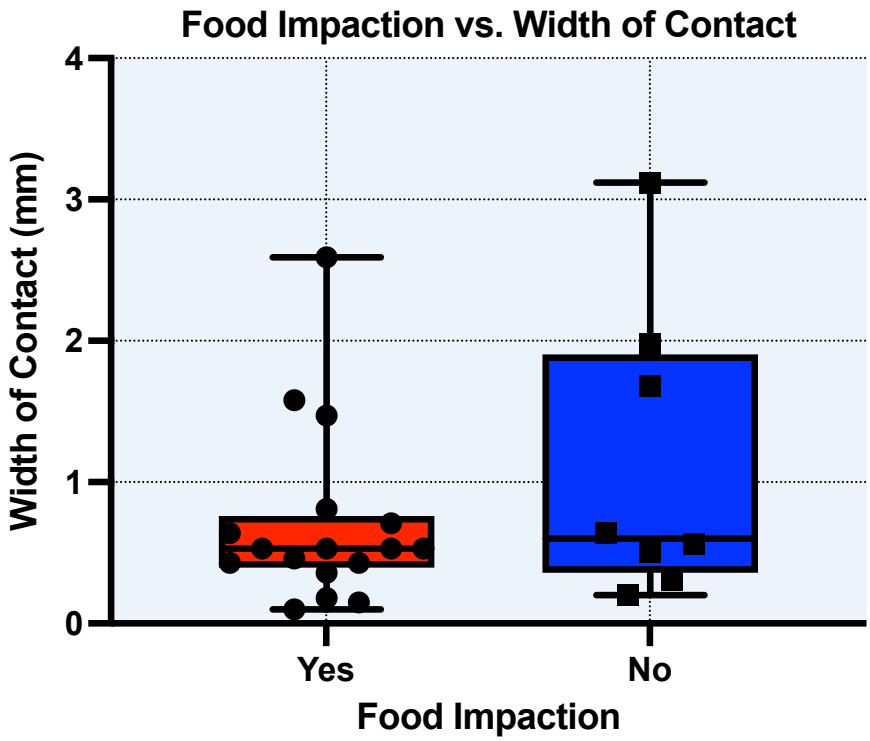

Figure 6: Food impaction vs. width of open contact

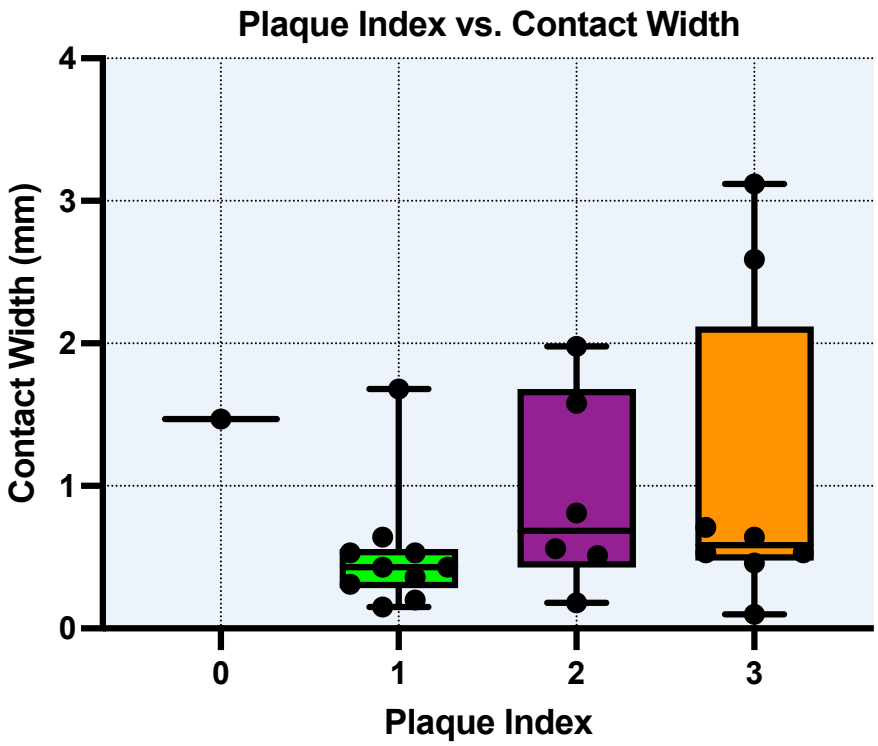

Figure 7: Plaque index vs. width of open contact

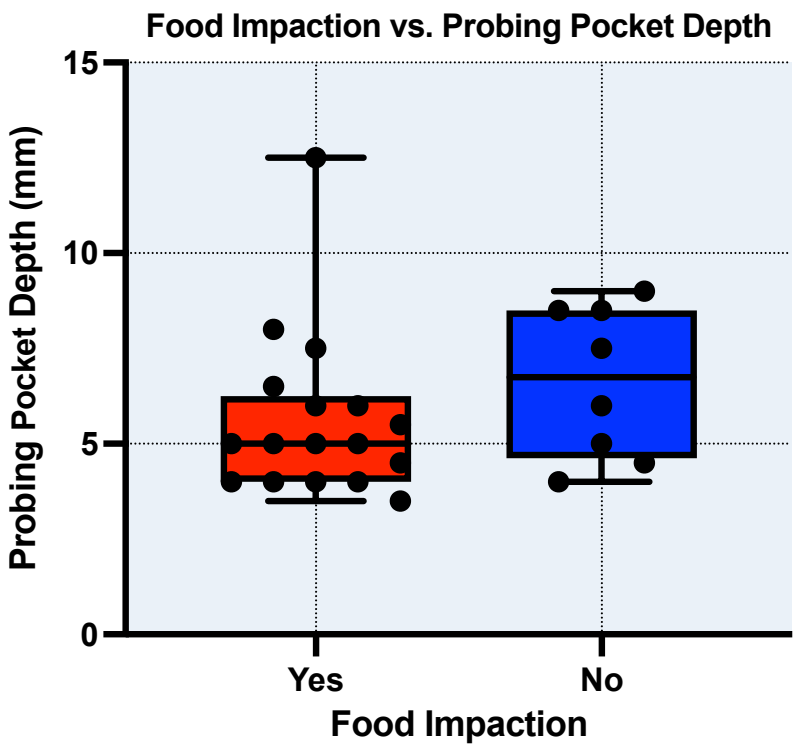

Figure 8: Food impaction vs. probing pocket depth

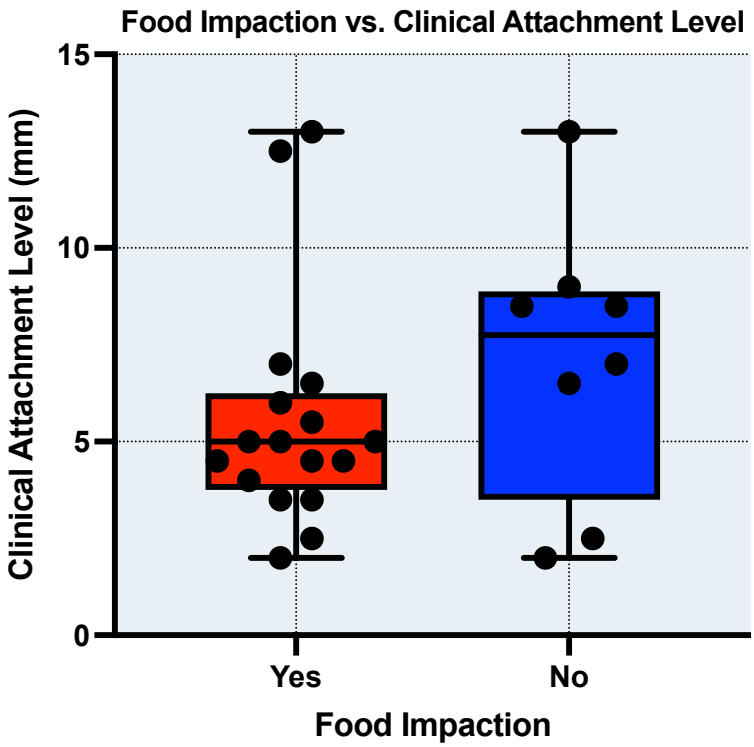

Figure 9: Food impaction vs. clinical attachment level

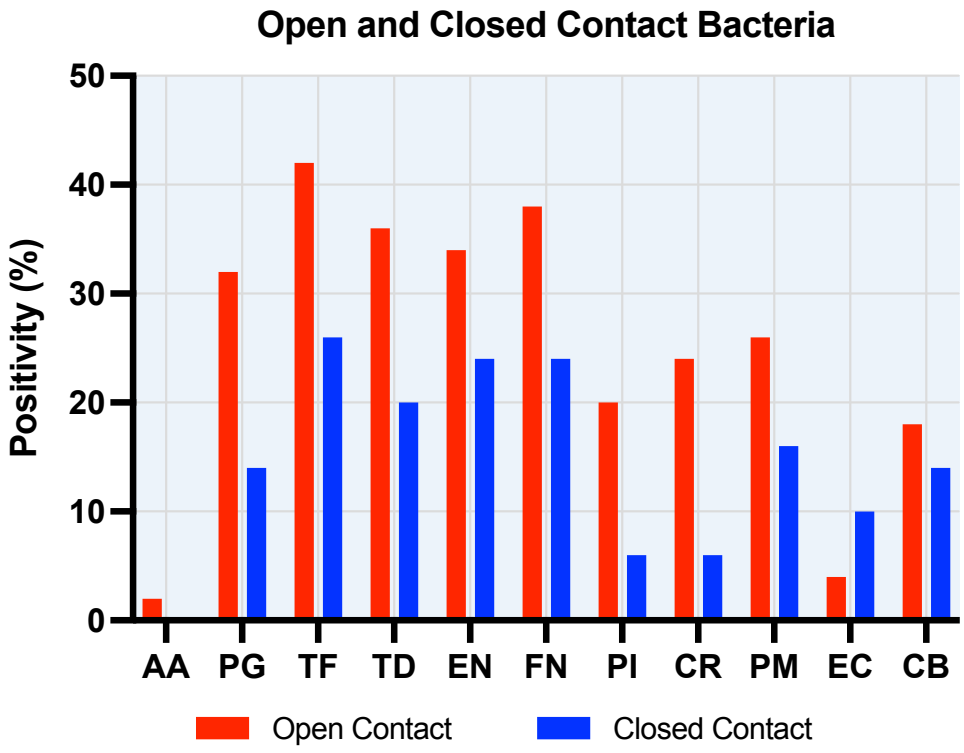

Figure 10: Bacteria present at open and closed contact sites

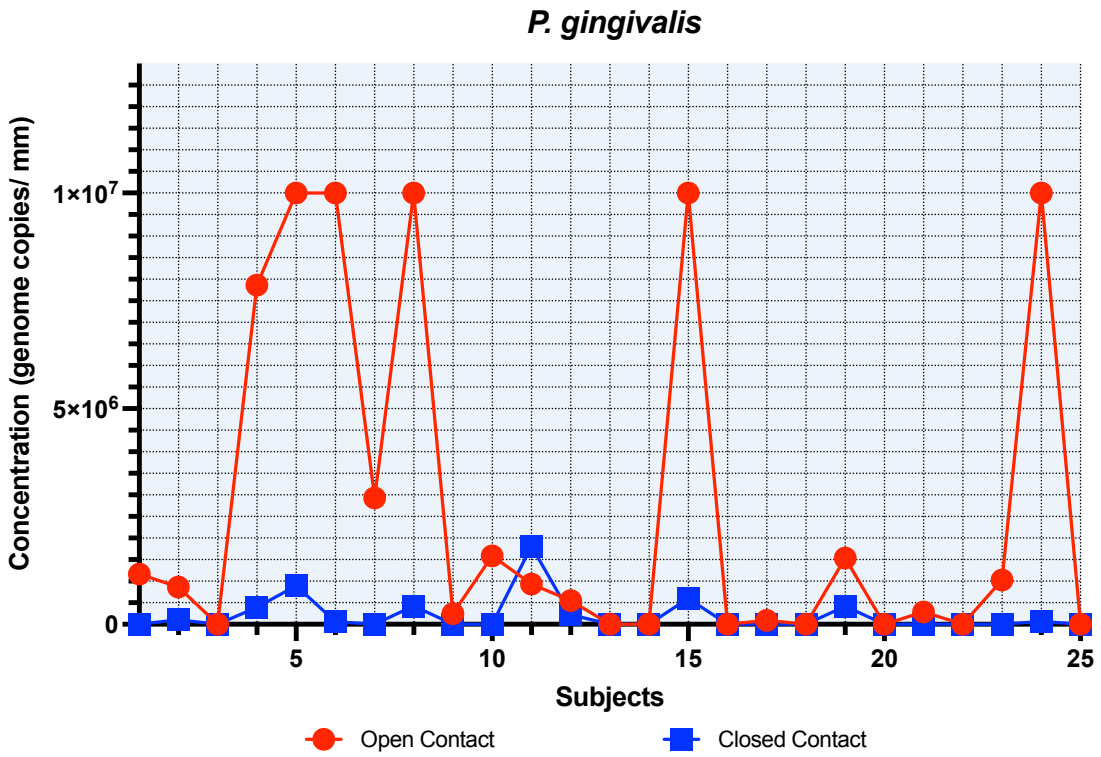

Figure 11: *P. gingivalis* concentration at open and closed contact sites.

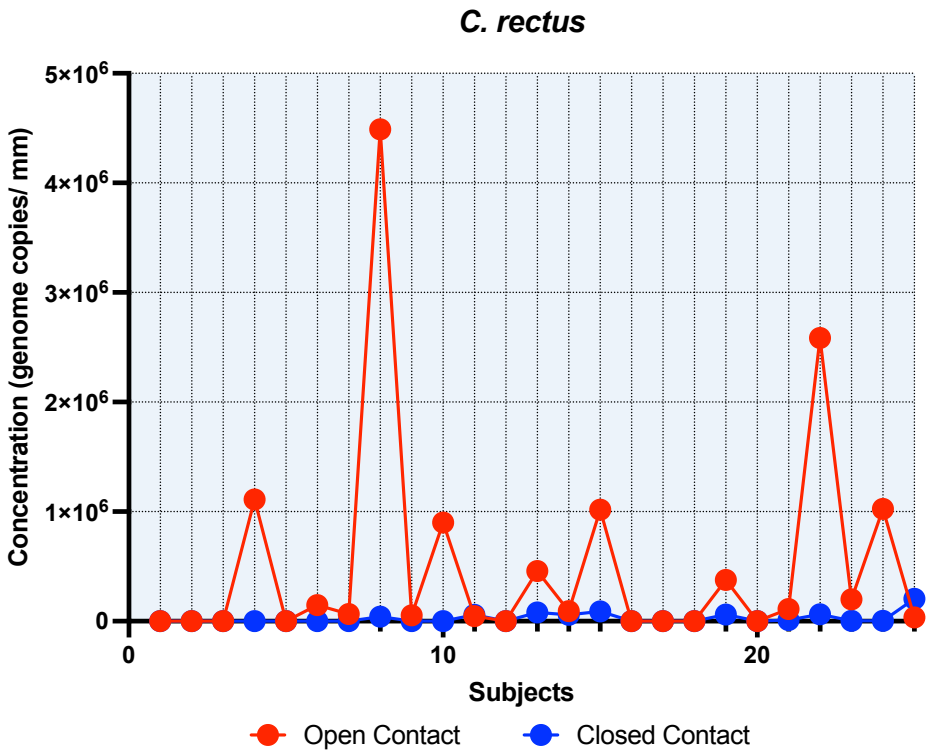

Figure 12: *C. Rectus* concentration at open and closed contact sites.

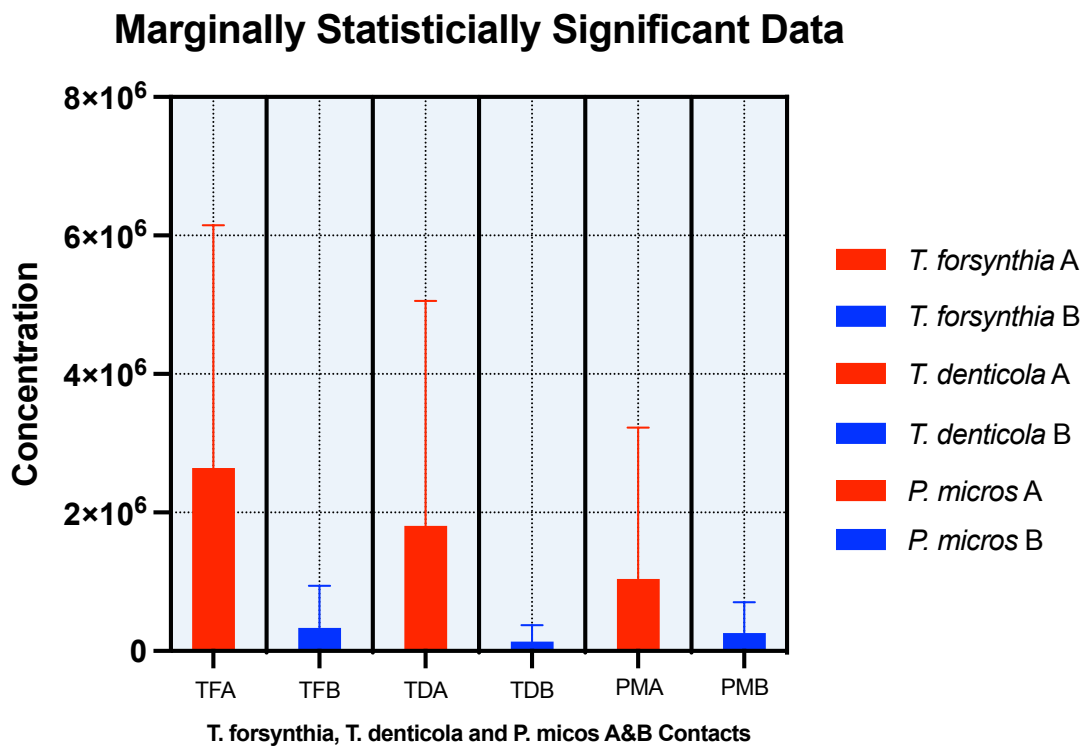

Figure 13: Marginally statistically significant data

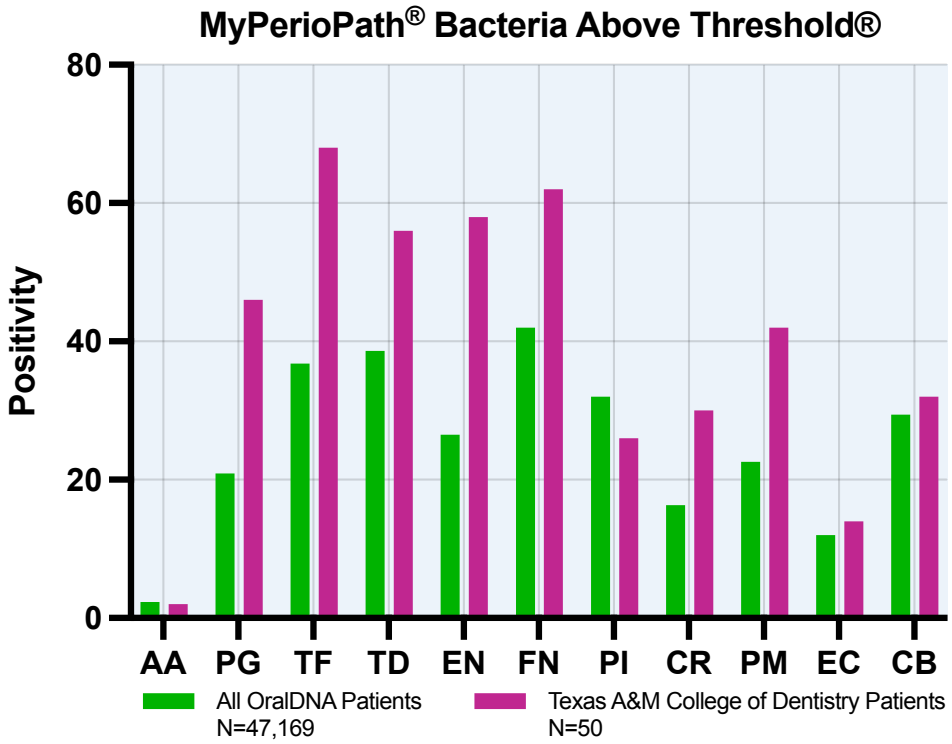

Figure 14: OralDNA® patients positivity for bacteria vs Texas A&M samples

TABLES

Variable	Contact site	Median, % or N	Interquartile range	P - value
Probing Pocket Depth (mm)	Open Closed	5 3.5	4.25, 7.5 3.0, 4.25	<0.001
Attachment Level (mm)	Open Closed	5 3	3.375, 7.75 1.75, 4.0	<0.001
Width of open contact (mm)	Open Closed	0.53 0	0.395, 1.140 0,0	-
Plaque Index	Open and Closed	2.0	1.0, 3.0	-
Bleeding on Probing (%)	Open Closed	92 74	- -	0.038
Food impaction (N)	Present Absent	17 8	- -	-

Table 1: Summary Statistics for Measurements at Open and Closed Contact Sites of Subjects (n=25)

Variable	Value	Food impaction	Plaque index
Probing depth (mm)	Rho P- value	-0.276 0.182	0.1733 0.4073
Attachment level (mm)	Rho p-value	-0.2803 0.175	0.238 0.252
Bleeding on probing	Rho p-value	0.168 0.421	-0.08 0.703

Table 2: Spearman correlations of side to side differences in probing depth, attachment level and bleeding on probing and differences in food impaction and plaque index.

Variable	Value	Probing Pocket Depth	Clinical Attachment Level	Bleeding on Probing	Plaque Index
Width of Open Contact (mm)	rho	0.5719	0.6281	-0.08	0.231
	p-value	**0.003**	**<0.001**	0.719	0.266

Table 3: Spearman correlations of Side to Side differences in probing depth, attachment loss, bleeding on probing, plaque index and the width of open contact.