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Composition of Microorganisms in Periodontal Pockets

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ABSTRACT

This pilot study investigated 73 healthy and diseased periodontal pockets evaluated by micropipette analysis to evaluate the bacteria existing in healthy and diseased pockets. The biofilm in the pockets are compared to 16 swish and 14 irrigation and swish saliva samples. Comparing the oral saliva samples (swish and irrigation/swish) pocket biofilm results to micropipette analysis of the healthy and diseased periodontal pockets enables an evaluation of consistency. Noteworthy differences were found. The patients came from two separate offices (one periodontist and one general dentist) and the evaluators were calibrated for reliability.

The patients were categorized as free of periodontal disease (PD) having 3 mm pockets or less without bleeding upon probing (BOP) and patients with periodontal disease having 3 mm pockets with BOP or 4 mm pockets or greater. Bacteria were collected by micropipette from the periodontal sulcus or pocket and were evaluated by DNA analysis (MicroGenDx) by pocket depth to the genus/species level. The bacterial classification compared type of bacteria by a response to Gram stain (Gram-positive or Gram-negative) and categorized the bacteria as; anaerobic, facultative anaerobes and aerobic. Fungi were also evaluated. A log computation of the number of microorganisms per volume was recorded.

Differences in the findings between the "swish" analysis compared to the "irrigation and swish" compared to the pocket micropipette analysis present conflicting results. The direct pocket analysis provides the best means of determining which bacteria predominate and/or co-exist in healthy and diseased patients' periodontal tissues. The predominance of type and category of bacteria and the changes from health to varying stages of disease are presented.

There is a shift from a more aerobic and facultative anaerobic Gram-positive biofilm in healthy pockets that are replaced by anaerobic and Gram-negative biofilm found in periodontal disease. The difference starts at the 3 mm pocket depth between patients without periodontal disease versus patients with periodontal disease. Treatment results can be appraised by comparing the microbiome components to that found in health as compared to components found in disease. This is a small sample and additional investigations may be needed to confirm the findings of this study.

Keywords: Microorganisms, Periodontal, Microbiome, Gram-negative

INTRODUCTION

Biofilms related to periodontal disease have been evaluated by various means. Research using a checkerboard DNA-DNA hybridization to identify 40 different bacterial strains shows the predominant initial colonizers of the oral environment are Gram-positive facultative anaerobic cocci and rods, including Streptococcus and Actinomyces species and aerobic bacteria [1]. These initial colonizers provide a foundation for further development of dental biofilm. Early microbial succession involved mainly Gram-positive and Gram-negative aerobic and facultative anaerobes with a few Gram-positive anaerobes [2].

It has been postulated that a shift occurs in the microbial concentrations as periodontal pockets are formed. The shift in the periodontal microbiome that accompanies an increase in Gram-negative anaerobic species is now accepted as an indicator of periodontal disease [3]. Uematsu and Hoshino [4] reported that approximately 90% of microorganisms isolated from periodontal pockets are strictly anaerobic and certain sets of bacteria have been frequently detected at elevated levels in periodontal lesions as compared with healthy tissues.

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These findings indicate that typical co-colonization of specific oral species, among which a cluster with the nomenclature "red complex" composed of the Gram-negative anaerobic species *Tannerella forsythia*, *P. gingivalis* and *Treponema denticola*, are associated with increased pocket depth and bleeding upon clinical pocket probing, Socransky et al. [5] report the other four clusters examined were not shown to be associated with clinical parameters indicating periodontal disease.

Other possible explanations about the shift from health to periodontitis involves when a low number of bacteria (10^2-10^3) that are mostly Gram-positive aerobic and facultative anaerobes increase in number and are overtaken by a greater number (10^4-10^5) of Gram-negative anaerobic microorganisms [6]. Gram-positive and Gram-negative bacteria equally induce IL-1 beta, but Gram-positive bacteria generate twice as much TNF-alpha. Gram-negative bacteria induce at least twice as much IL-6 and IL-8 [7]. The increased incidence of Gram-negative anaerobic bacteria induces systemic challenges and immune system responses.

Gram-negative bacteria produce lipopolysaccharides (LPS) that induce inflammatory cell infiltrate in the blood vessel walls, causing vascular smooth muscle proliferation, vascular fatty degeneration and intravascular coagulations. LPS up-regulates endothelial cell adhesion molecule expression and increases the secretion of interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-alpha) and thromboxane, which increases platelet aggregation and adhesion, causing the formation of lipid laden foam cells and deposits of cholesterol and cholesterol esters [8].

The severity of the host response to both Gram-negative and Gram-positive bacteria plays a major role in causing inflammation and tissue sepsis. These bacteria produce a range of virulence factors that enable them to escape the immune defenses and disseminate to remote organs. Toxins that interact with host cells via specific receptors on the cell surface trigger a dysregulated immune response [9]. Gram-negative bacteria pose more host inflammatory complications due to:

1. There is a membrane present around the cell wall of gram-negative bacteria which increases the risk of toxicity to the host, but this membrane is absent in gram-positive bacteria.
2. Porin channels are present in gram-negative bacteria which can prevent the entry of harmful chemicals and antibiotics like penicillin. These channels can also expel out antibiotics making it more difficult to treat in comparison to gram-positive bacteria.
3. The risk of resistance against antibiotics is higher in Gram-negative bacteria due to the presence of external covering around the cell wall.
4. Gram-negative bacteria possess both exotoxins and endotoxins but in case of gram-positive bacteria there are individual exotoxins [10].

Another possible explanation of the shift in the biofilm relates to oxygen levels in the periodontal pockets. A relationship exists between the subgingival microbes and the oxygen tension in periodontal pockets, suggesting anaerobes increase as the pocket depth increases and oxygen tensions decrease [11]. Anaerobic bacteria are shown to be resistant to short-term periodontal therapy [12] and can regrow in a matter of days, continuing the infectious process [13].

Critical wound colonization is a term utilized to express wound chronicity as it relates to the quantity and quality of the infectious agents as well as the host responses [14]. An increased number of more virulent bacteria commonly relates to an increase in the host inflammatory responses. This involves a framework where virulent immune provoking behaviors and enhanced immune resistance enables invading pathogens to overcome resident microorganisms [15]. Pathogenic bacteria succeed by creating a novel immunologic challenge to which they are already adapted. Decreasing the number of pathogens reduces virulence, while specific bacteria are associated with higher virulence [16].

Oral saliva diagnostics provide information on a variety of pathogens, but many of these systems are limited in scope and whether the bacteria originate from the periodontal tissues or other oral structures is questioned. There are no FDA-approved salivary diagnostic tests for evaluating the risk of periodontal disease [17]. Site specific diagnostics can determine the pathogens present in the periodontal infection and this more precise information may be essential to customize the proper corrective measures and determine treatment results.

This article discusses the biofilm constituents that appear from oral saliva analysis compared to the biofilm present in the periodontal pocket by micropipette analysis. There is a discrepancy between the findings between a saliva analysis and a micropipette analysis. These differences may have a bearing on treatment options and determining treatment success or failure.

METHOD

Three methods of biofilm analysis were compared in this study. All evaluators were calibrated for reliability and accuracy. Oral saliva analysis, lavage and oral saliva analysis and direct pocket pathogen analysis by micropipette collection are examined by DNA analysis to determine bacterial presence in healthy and diseased periodontal pockets. The results of these different analysis systems were also compared. This pilot study involves 73 periodontal sulcus samples collected by micro-pipette, 14 (lavage and swish) and 16 (swish only) samples submitted for DNA analysis (MicroGenDx) to evaluate the composition and

characteristics of the biofilm. Only the bacteria and fungi that comprise 2% or more of the periodontal biofilm were identified. Partial percentages are rounded off to the closest whole number.

Patients were divided into those without periodontal disease (3 mm pockets or less with no BOP) and those with periodontal disease (3 mm or more with bleeding). The DNA analysis provided the percentage and number of Gram-positive versus Gram-negative bacteria and whether the bacteria were anaerobic, facultative anaerobes or aerobic. Fungi were also reported.

The compositions of biofilms in healthy patients were evaluated by micropipette analysis to determine what bacteria comprise the biofilm in healthy pockets. These findings were compared to the biofilm found in diseased patient's pockets to determine possible patterns of pathogenicity. The biofilm determined by micropipette collection were compared to oral saliva analysis. Two means of saliva analysis were utilized; "swish" saliva samples of the entire mouth and a second irrigation of the periodontal pockets, swish and saliva collection to evaluate the biofilm.

A swish only saliva collection and a lavage and swish saliva collection were completed and all bacteria and fungus 2% or more were computed as well as the number of bacteria/volumes. Samples that are found 1% of the time or less are not compared, so results will often demonstrate less than 100% in the evaluation.

In the "swish" sample, 5 cc of sterile saline was swished in the patient's mouth for 60 seconds and then collected for analysis in a sterile container. In the second salivary analysis, 5 cc of sterile saline was placed into an irrigation syringe which was used to lavage the periodontal pockets. This irrigation-swished material was swished and maintained in the patient's mouth for 60 s and then expectorated into a sterile container for shipment for biofilm analysis.

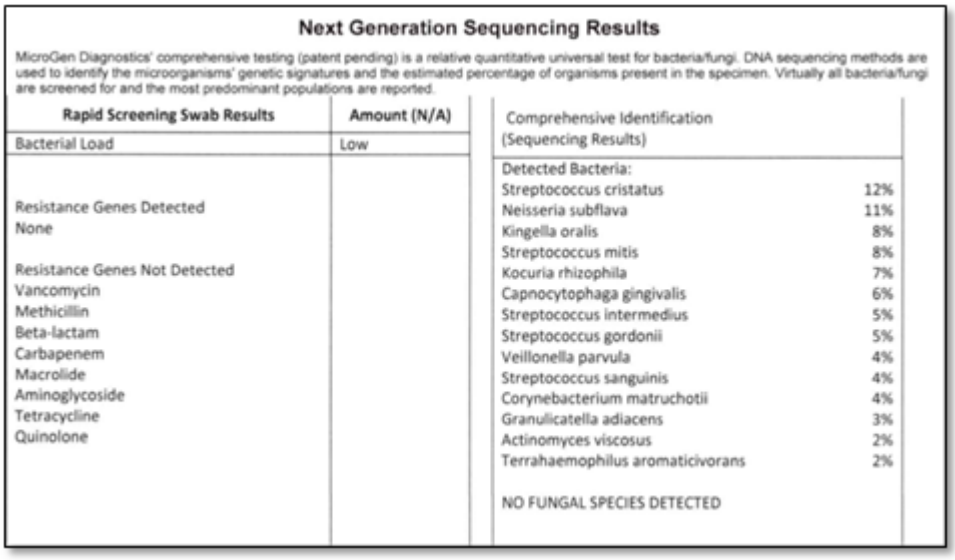
Individual periodontal pockets were analyzed by micro-pipette suction to remove the biofilm from periodontal sulcus or pocket. A blunt tipped needle attached to a syringe was inserted to the depth of the periodontal pocket and the syringe plunger was slightly elevated for 10 seconds to create a negative pressure within the syringe, so the microbiome was "sucked" into the tip. The tip was removed from the pocket with the plunger elevated so all the sample remained in the tip. The micro-pipette tip was removed from the syringe and placed in a sterile labeled transport container for shipment to MicroGenDx for analysis of the biofilm.

MicroGenDx evaluated all samples as to the composition of the biofilm. All samples were found to contain adequate biofilm for analysis. MicroGenDx recorded the concentration of the biofilm as low ($10X^{3-5}$), medium ($10\ X^{5-7}$) and high ($10\ X^{7>}$). The numeric compositions used to determine the bacteria/volume were computed as $10X^3$, $10X^5$ and $10X^7$. All microorganisms that comprise 2% of the population or greater were recorded. Comparisons were made between healthy pockets (pocket probing depths 3 mm or less without BOP) that were compared to patients clinically determined to have periodontal disease.

Multiple bacteria were found in this study and divided into distinct groupings: Gram positive or Gram negative. The samples were also categorized as fungi or types of bacteria: anaerobes, facultative anaerobes and aerobic bacteria to the genus/species level. Subspecies were not determined. The number of bacteria was also evaluated to determine the population density per volume according to the sample technique.

The oral solutions "swish and expectorate" was gathered first. Second the oral rinse solution was irrigated into the deeper periodontal pockets and swished and collected. Micro-pipette samples of periodontal pockets were gathered after the saliva samples were collected. All samples were labeled and shipped for analysis.

All samples were found to contain a minimum of $10X^3$ or greater. The type of bacteria and fungi were evaluated for different pocket depths and compared to the oral saliva sample techniques. The following is an example of a MicroGenDx report **(Figure 1)**.



Next Generation Sequencing Results

MicroGen Diagnostics' comprehensive testing (patent pending) is a relative quantitative universal test for bacteria/fungi. DNA sequencing methods are used to identify the microorganisms' genetic signatures and the estimated percentage of organisms present in the specimen. Virtually all bacteria/fungi are screened for and the most predominant populations are reported.

Rapid Screening Swab Results	Amount (N/A)
Bacterial Load	Low
Resistance Genes Detected	
None	
Resistance Genes Not Detected	
Vancomycin	
Methicillin	
Beta-lactam	
Carbapenem	
Macrolide	
Aminoglycoside	
Tetracycline	
Quinolone	

Comprehensive Identification (Sequencing Results)	
Detected Bacteria:	
Streptococcus cristatus	12%
Neisseria subflava	11%
Kingella oralis	8%
Streptococcus mitis	8%
Kocuria rhizophila	7%
Capnocytophaga gingivalis	6%
Streptococcus intermedius	5%
Streptococcus gordonii	5%
Veillonella parvula	4%
Streptococcus sanguinis	4%
Corynebacterium matruchotii	4%
Granulicatella adiacens	3%
Actinomyces viscosus	2%
Terrahaemophilus aromaticivorans	2%
NO FUNGAL SPECIES DETECTED	

Figure 1. MicroGenDx sequencing results.

The percent number of the individual type (genus/species) was first categorized as Gram positive versus Gram negative. The sample was further analyzed by the percentage of anaerobes, facultative anaerobes and aerobic bacteria. Bacteria that were less than 2% of the population were not considered in the evaluations. In the above sample no fungi were discovered.

The percentage presence of Gram positive bacteria in the above example are the Streptococcus, Kocuria, Granulicartella and Actinomyces, comprising 42% of the population. Gram negative species (remaining) comprise 39% of the population with the remaining 19% existing in less than 2% concentrations. Anaerobes, facultative anaerobes and aerobic percentages of the total population would be: 19%, 41% and 21%. No fungi were present in this sample. The percentages of the specific categories may be depicted in graphic representation (**Figure 2**).

Yellow	Gram positive
Pink	Gram negative
Red	Anaerobes
Green	Facultative anaerobes
Blue	Aerobes
Black	Fungi

The graphic representation of the MicroGenDx sample above is illustrated in **Figure 2**.

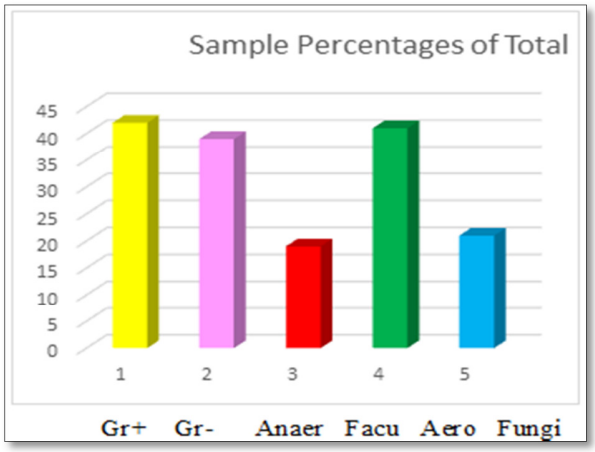


Figure 2. It is an illustration in bar graph form to demonstrate the biofilm in the sample. These illustrate 42% of the sample are Gram-positive and 39% are Gram-negative. 19% are anaerobes, 41% are facultative anaerobes and 21% are aerobic bacteria. The bacteria not represented are bacteria that were found in less than 2% of the total population. No fungi are present in this sample.

RESULTS

The results of this study help determine the microbes found in healthy tissues and evaluate changes in the periodontal microbiome as periodontal pocket depth and pathology increase. The initial evaluations are completed for patients without periodontal disease (PD) and where no pockets are greater than 3 mm. The initial tests involved an oral "swish" **(Figure 3)**, "irrigation and swish" **(Figure 4)** and a micropipette analysis **(Figure 5)** of the microbiome.

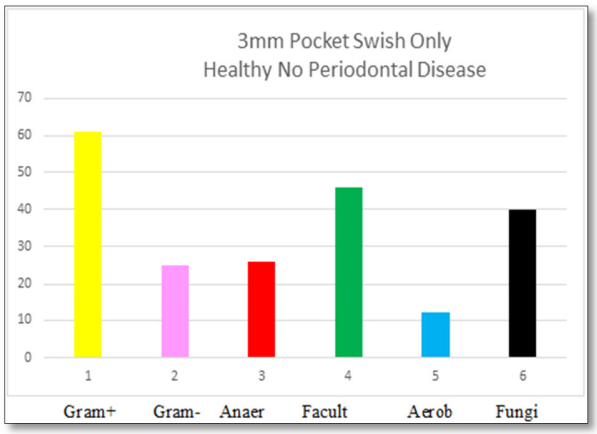


Figure 3. Swish analysis for normative periodontal patients.

The "swish" samples of 3 mm pockets for patients free of periodontal disease are presented in **Figure 3**. The response to a Gram stain divides the sample into two types of microorganisms, those which are Gram-positive and those which are Gram-negative. Only those bacteria present 2% of the time are greater are included in this and all following analyzes.

Gram-positive bacteria are present at 61% and Gram-negative are present at 25%. The categories of bacteria found are anaerobes at 26%, facultative anaerobes as the predominant species at 46% and aerobic bacteria at 12%. Fungi are found in 40% of the patient's "swish" samples. The number of bacteria/volume is 10 × 5.8. These findings can be compared to the "irrigation and swish" samples for periodontal healthy patients 3 mm pockets which are presented in **Figure 4**.

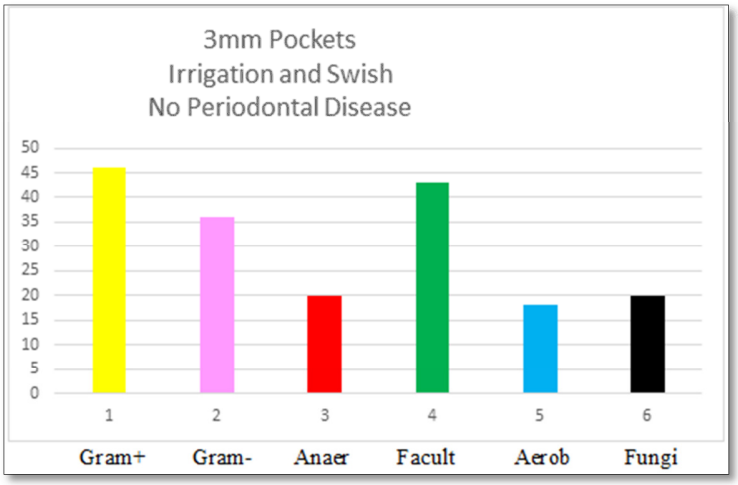


Figure 4. Irrigation and Swish analysis for normative periodontal patients.

The "irrigation and swish" samples for healthy patients demonstrate Gram-positive bacteria are present 46% compared to 36% for Gram-negative bacteria. The categories of bacteria are 20% anaerobic, 43% facultative anaerobes and 18% aerobic bacteria with a 20% incidence of fungi. The number of bacteria/volume is 10 × 5.5. These results are compared to the bacteria found in the healthy periodontal sulci by micropipette analysis.

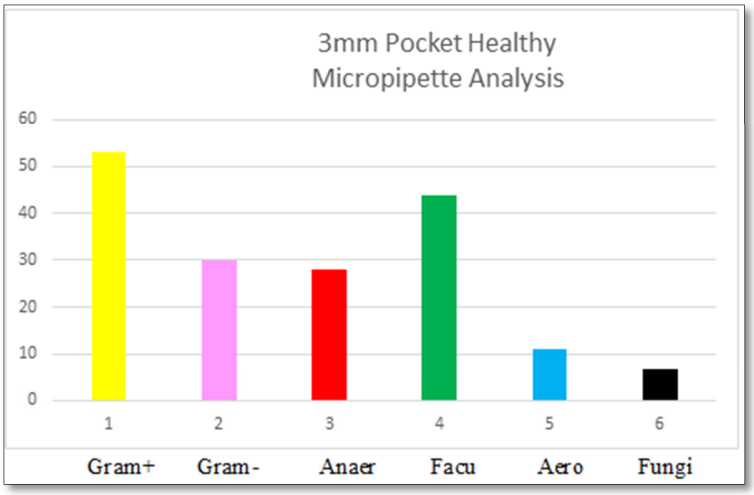


Figure 5. Micropipette analysis for healthy periodontal patients.

Figure 5 demonstrates the bacteria found in 3 mm periodontal pockets for patients without periodontal disease. Gram- positive bacteria are present at 53% and are more prevalent than Gram-negative bacteria at 30%. There are more facultative anaerobes 44% than anaerobes 28%, with aerobic bacteria present at 11%. Fungi are present at 7%. The number of bacteria is 10 × 4.0.

Table 1 compares the microorganisms found in the saliva "swish" sample, "irrigation and swish" sample and the micropipette analysis samples for patients without periodontal disease. The predominant types of bacteria in all samples are Gram-positive bacteria. All three samples demonstrate the predominant category of bacteria is facultative anaerobes, followed by anaerobic bacteria, and aerobic bacteria. Fungi are evident in the both oral saliva samples and in the micropipette analysis at varying proportions. There is more bacteria/area in the saliva samples as compared to the micropipette analysis. The predominant species in healthy periodontal tissues and in the oral saliva samples of healthy periodontal patients are Gram-positive and facultative anaerobes with a lesser percent presence of anaerobes and aerobic bacteria and some fungi.

Table 1. Comparison of "swish", "irrigation and swish" and micropipette analysis for 3 mm pockets for patients free of periodontal disease.

	Swish	Irrigation and Swish	Micropipette
Gram-positive bacteria	61%	46%	53%
Gram-negative bacteria	25%	30%	30%
Anaerobic bacteria	26%	20%	28%
Facultative anaerobes	46%	43%	44%
Aerobic bacteria	12%	18%	11%
Fungi	40%	20%	7%
Number of bacteria/volume	10 × 5.8	10 × 6.0	10 × 4.0

Three-millimeter pockets are accepted as normal regarding periodontal health [18]. Patients with periodontal disease somewhere in their mouth also have 3 mm pockets. The composite of the biofilm of patients 3 mm pockets with periodontal disease are analyzed by "swish", "irrigation and swish" and micropipette analysis of 3 mm pockets for patients with periodontal disease are presented in **Figure 6**.

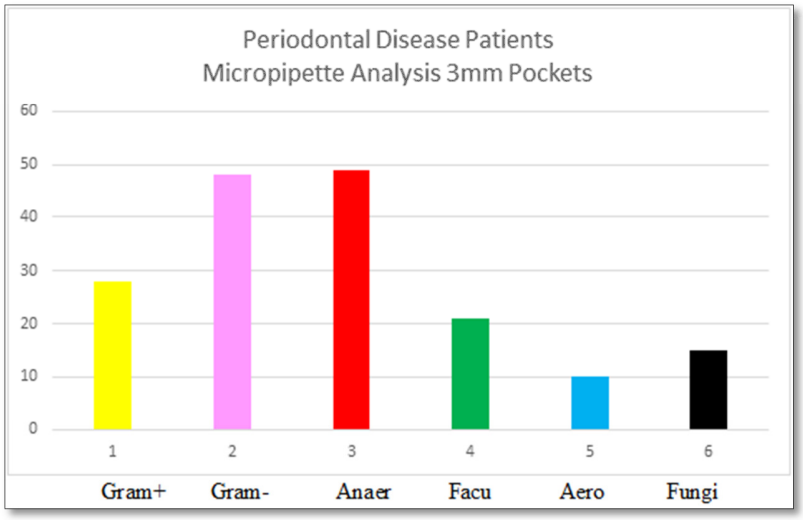


Figure 6. Micropipette analysis for diseased periodontal patients.

Figure 6 graphically represents bacteria presence in 3 mm periodontal pockets from patients diagnosed with periodontal disease somewhere in their mouth by micropipette analysis. Gram positive microbes are present at 27% as compared to Gram-negative present at 50%. Anaerobes constitute 48% of the population, facultative anaerobes comprise 20% and aerobic bacteria are present 11% of the time. Fungi are found at 15%. The number of bacteria is 10 × 3.6.

There are distinct differences between the microbiome in healthy 3 mm periodontal pockets and 3 mm periodontal pockets from patients with periodontal disease. These differences are presented in **Table 2**. **Table 2** compares the bacteria present in 3 mm pockets of patients free of periodontal disease to the bacteria found in 3 mm pockets of patients who have periodontal disease somewhere in their mouth.

Table 2. Comparison of micropipette analysis for 3 mm pockets for patients with periodontal disease.

	Micropipette 3 mm Healthy	Micropipette 3 mm PD
Gram-positive bacteria	50%	27%
Gram-negative bacteria	30%	50%
Anaerobic bacteria	28%	48%
Facultative anaerobes	44%	20%
Aerobic bacteria	11%	11%
Fungi	7%	15%
Number of bacteria/volume	10 × 4.0	10 × 3.6

Table 2 demonstrates differences in the microbiome by micropipette analysis found in 3 mm pockets of patients with and without periodontal disease. Gram-positive bacteria predominate in healthy 3 mm pockets, while Gram-negative bacteria predominate in 3 mm pockets of periodontal disease patients. Facultative anaerobes are the predominant species in healthy 3 mm periodontal pockets, while anaerobes predominate in 3 mm periodontal pockets of patients with periodontal disease. Aerobic bacteria remain constant for both groups and a small percentage of fungi are found in health 3 mm pockets and in 3 mm pockets of periodontal disease patients.

Periodontal pockets greater than 3 mm are determined in this study to be evidence of periodontal disease. These are evaluated by oral saliva "swish", "irrigation and swish" samples and micropipette analysis. Oral saliva samples "swish" and "irrigation and swish" are taken for all patients found to have periodontal disease. The composite of the oral saliva findings are presented in **Figure 7** "swish" and **Figure 8** "irrigation and swith" sample results.

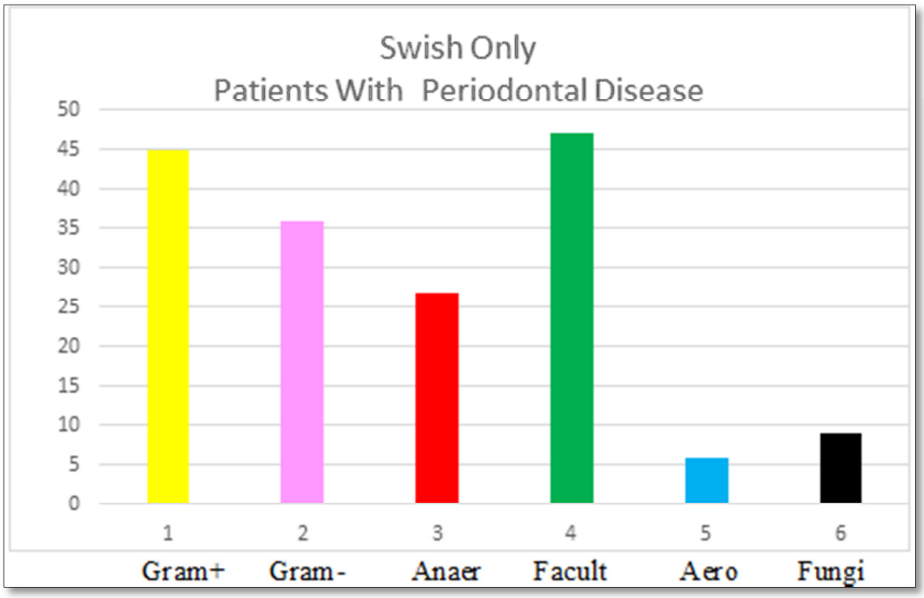


Figure 7. Swish saliva analysis of periodontal disease patients.

Figure 7 demonstrates the composition of the biofilm found in oral saliva "swish" analyzes for patients with periodontal disease. Gram-positive bacteria are evident at 45% and are more prevalent than Gram-negative bacteria at 36%. Facultative anaerobes predominate at 47% with anaerobes at 27% and aerobic bacteria at 6%. Fungi are present at 9%. The number of bacteria per volume is 10×6.6.

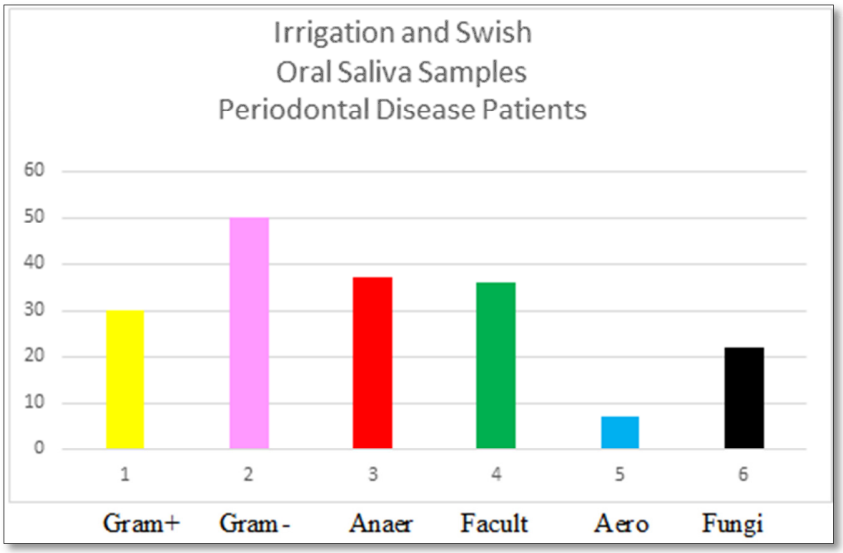


Figure 8. Irrigation and Swish saliva analysis of periodontal disease patients.

Figure 8 demonstrates the biofilm composition of oral saliva samples using the "irrigation and swish" technique. Gram-positive bacteria are found at 30% and Gram-negative bacteria are found at 50%. 20% of the bacteria are present in concentrations of less than 2%. Anaerobes are found at 37% with facultative anaerobes at 36% and aerobic bacteria at 7%. Fungi are present at 22%. The number of bacteria per volume is 10×6.8.

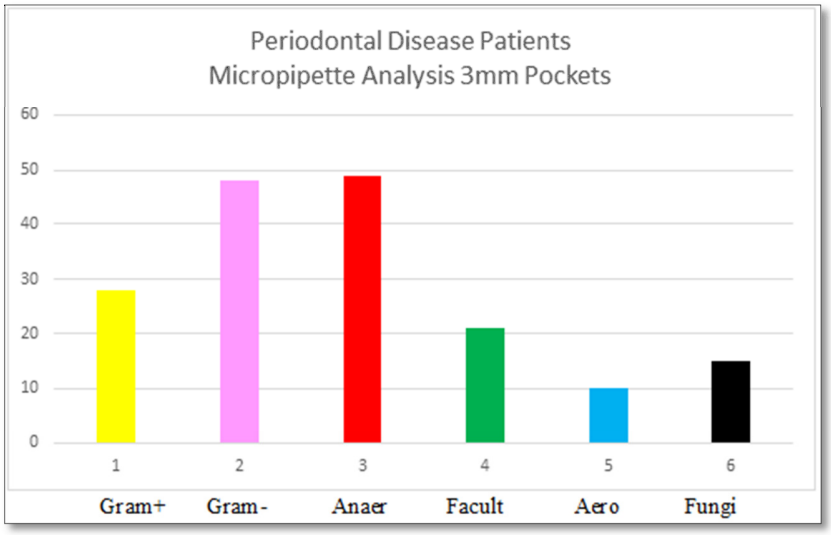


Figure 9. Analysis of periodontal disease patients.

Figure 9 demonstrates the bacteria found in 3 mm pockets of patients with periodontal disease somewhere in their mouth. The types of bacteria are 27% Gram-positive and 50% are Gram-negative. The categories of bacteria are 48% are anaerobic, 20% are facultative anaerobes and 11% are aerobic bacteria. Fungi are present at 15%. The number of bacteria/volume is 10 × 3.6.

Differences are evident in these findings. The oral "swish" analysis demonstrates Gram-positive and facultative anaerobes are the predominant species in patients with periodontal disease. The "irrigation and swish" analysis demonstrates Gram-negative bacteria and an almost equal number of facultative anaerobes and anaerobes predominate. The micropipette analysis of 3 mm periodontal pockets from patients with periodontal disease demonstrates Gram-negative and anaerobic bacteria predominate.

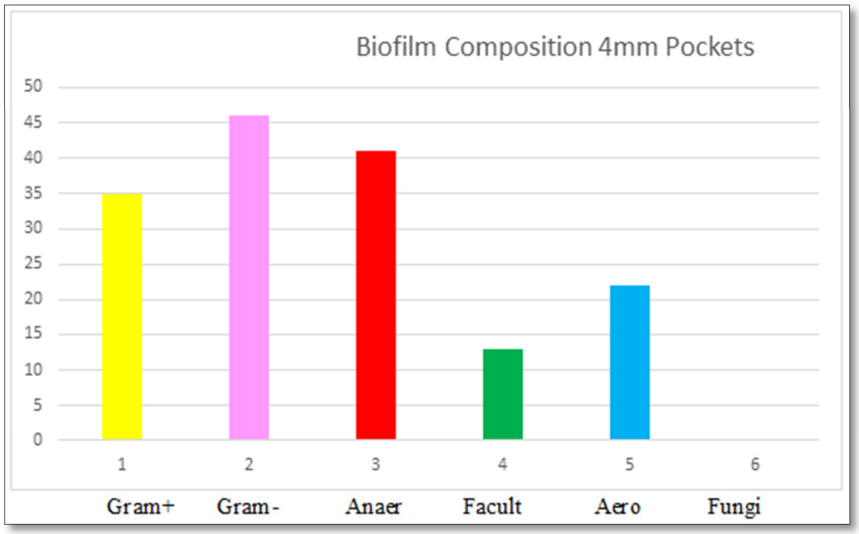


Figure 10. Micropipette analysis of the biofilm in 4 mm pockets.

Figure 10 demonstrates the bacteria found by micropipette analysis from patients 4 mm periodontal pockets. The type of bacteria are 35% Gram-positive and 46% Gram-negative. The categories of bacteria are 41% are anaerobic, 13% are facultative anaerobes and 22% are aerobic bacteria. No fungi are found in 4 mm pockets. The number of bacteria per volume is 10 × 4.3.

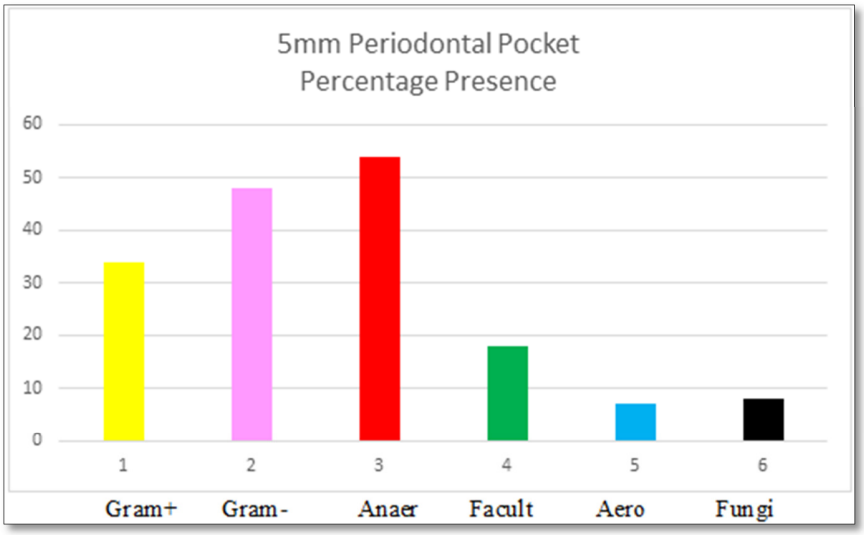


Figure 11. Micropipette analysis of the biofilm in 5 mm pockets.

Figure 11 demonstrates the bacteria found by micropipette analysis from patient's 5 mm periodontal pockets. The type of bacteria are 34% Gram-positive and 48% Gram-negative. The categories of bacteria are: 54% are anaerobic, 18% are facultative anaerobes and 7% are aerobic bacteria. Fungi are found at 8% in 5 mm pockets. The number of bacteria per volume is 10×4.8.

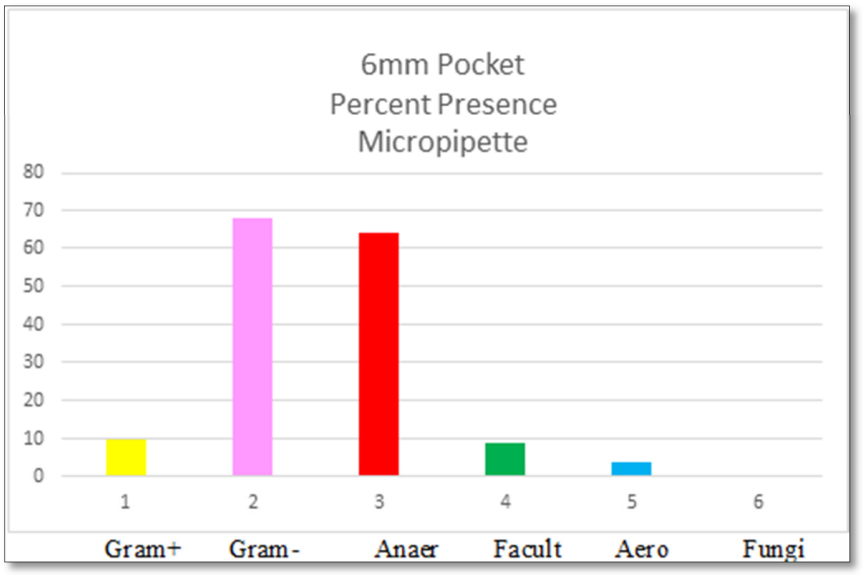


Figure 12. Micropipette analysis of the biofilm in 6 mm pockets.

Figure 12 demonstrates the bacteria found by micropipette analysis from patients 6 mm periodontal pockets. The types of bacteria are: 10% Gram-positive and 68% Gram-negative. The categories of bacteria are: 64% are anaerobic, 9% are facultative anaerobes and 4% are aerobic bacteria. Fungi are absent from 6 mm pockets. The number of bacteria per volume is 10×4.7.

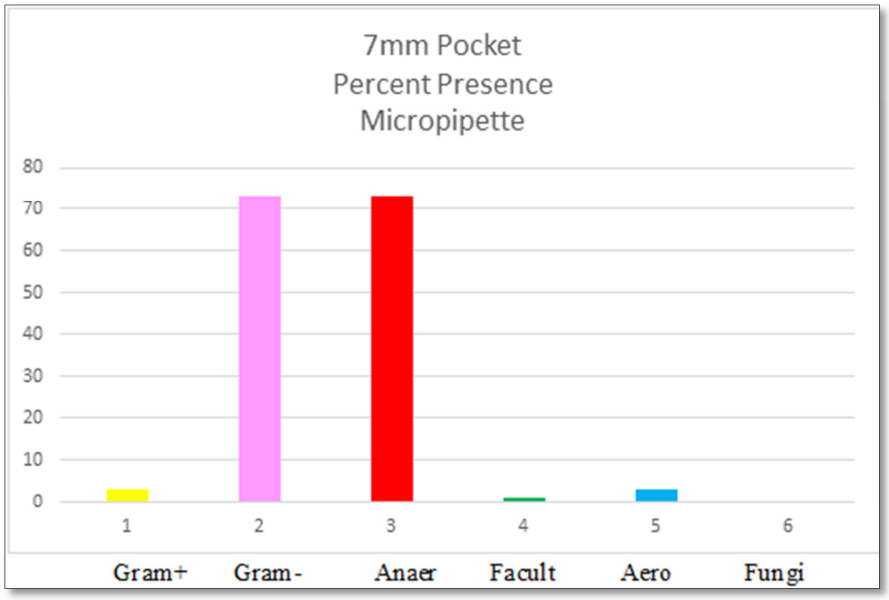
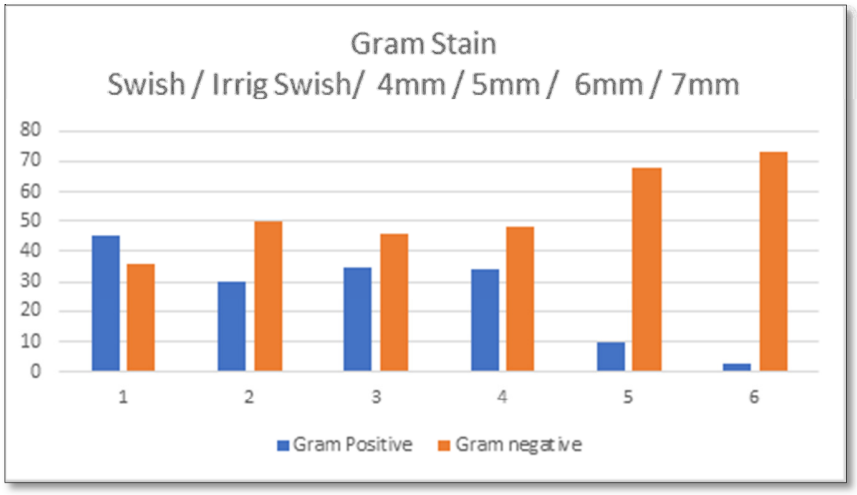


Figure 13. Micropipette analysis of the biofilm in 7 mm pockets.

Figure 13 demonstrates the bacteria found by micropipette analysis from patients 7 mm periodontal pockets. The types of bacteria are: 3% Gram-positive and 73% Gram-negative. These are the bacteria that comprise at least 2% of the total biofilm consistency. The categories of bacteria are: 73% are anaerobic, 1% is facultative anaerobes and 3% are aerobic bacteria. Fungi are absent from 7 mm pockets. The number of bacteria per volume is 10×4.6.

Comparison of the type of bacteria and category of bacteria for the oral saliva analyzes "swish", "irrigation and swish" and the microbiome found by micropipette analyzes for all periodontal pockets from periodontal disease patients are presented in **Figures 14A and 14B**.



Figures 14A. Swish, irrigation swish and micropipette analysis of 4, 5 6 and 7mm pokets.

Gram-positive bacteria are the predominant species in the oral "swish" method of evaluation. Gram-negative bacteria are the predominant species in the oral "irrigation and swish" method of evaluation. The micropipette analysis demonstrates Gram-negative bacteria are the predominant species in the 4-7 mm pockets and the incidence increases as the pocket depth increases.

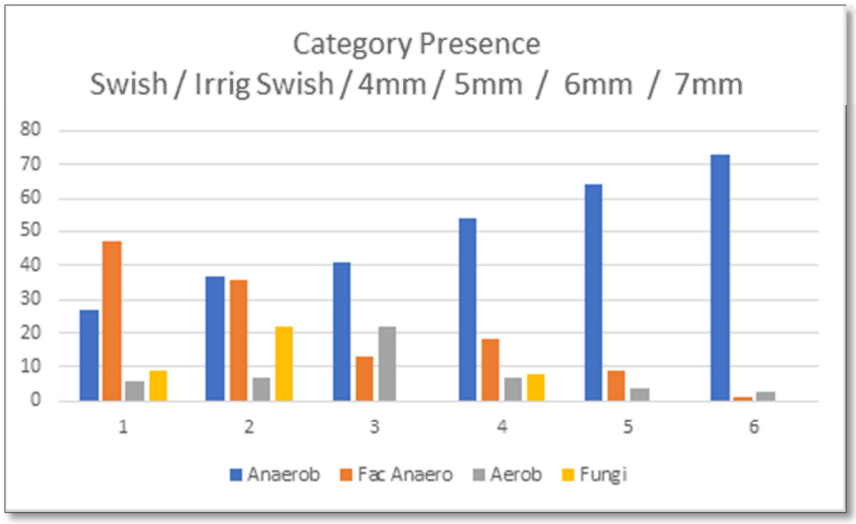


Figure 14B. Category presence of all bacteria in swish, irrigation and swish and micropipette analyses.

Figure 14B demonstrates that facultative anaerobes are the predominant species in the oral "swish" analysis. The predominant species in the "irrigation and swish" are facultative anaerobes and anaerobic bacteria. The predominant category of microbes in 4-7 mm pockets are anaerobic bacteria that increase in incidence as the pocket depth increases.

Figures 14 A and 14B illustrates the type and category of bacteria present in each sample for patients with periodontal disease. The oral saliva "swish" sample, "irrigation and swish" and the micropipette constituents for periodontal pockets of 4, 5, 6 and 7 mm are compared to evaluate similarities or differences. There is a significant difference in the "swish" analysis as the predominant species are Gram-positive bacteria, where the "irrigation and swish" and the micropipette analyzes demonstrate the predominant species are Gram-negative. The micropipette results for 4-7 mm pockets demonstrate the increased predominance of Gram-negative bacteria and the decrease in Gram-positive bacteria as the pocket depth increases.

Figure 14B demonstrates the "swish" analysis predominant category of bacteria is facultative anaerobes, where the "irrigation and swish" predominate category is facultative anaerobes and anaerobic bacteria. The micropipette analysis of 4-7 mm pockets predominant category demonstrates a steady increase in the anaerobic bacteria as the facultative anaerobic and aerobic bacteria decrease. The discrepancy between the findings between the "swish" analysis, "irrigation and swish" and the micropipette analyzes raise concerns as the different analyzes demonstrate difference predominant species and types of bacteria but were gathered from the same patients.

DISCUSSION

Knowing what comprises the biofilm in healthy periodontal tissues is important. The healthy biofilm serves as a treatment goal. Understanding the changes that occur in disease helps clarify disease etiology. The evaluation means to make these determinations must be consistent and accurate. There is a similarity of diagnostic results between oral saliva analyzes "swish" irrigation and "swish" and micropipette analysis when evaluating healthy periodontal tissues. The analyses find a predominance of Gram-positive and facultative anaerobes in healthy conditions.

The similarities diverge when evaluating periodontal disease tissues with the "swish", "irrigation and swish" and a micropipette analysis. The predominant species in periodontal disease oral saliva "swish" analysis are Gram-positive and facultative anaerobes. The predominant species in the oral saliva "irrigation and swish" analysis are Gram-negative anaerobic and facultative anaerobes. The predominant species in the micropipette analysis of 4-7 mm pockets shows an increasing incidence of Gram-negative and anaerobic bacteria. The difference between the three analysis techniques raises a question of accuracy and reliability.

One reason for the differences is the area evaluated. The "swish" analysis samples the entire oral area, resulting in bacteria from periodontal tissues, but also bacteria that are found on all other oral structures. The "irrigation and swish" sampling lavages the periodontal pocket, but also collects the bacteria from all other oral structures. The micropipette analysis only evaluates the microbiome of the periodontal pocket. The consistency of the micropipette results supports this method as the most accurate representation of the biofilm in the periodontal pocket.

The differences in predominance of the type and category of bacteria in the micropipette analysis begins in 3 mm pockets

for patients with periodontal disease as compared to 3 mm pockets of patients without periodontal disease. The micropipette microbiome in the diseased pocket demonstrates an increasing incidence of Gram-negative and anaerobic bacteria as the pocket depth increases.

Evaluation of treatment success may be misleading with the differences of the three methods. When evaluating periodontal disease patients, the "swish" analysis demonstrates a predominance of Gram-positive and facultative anaerobe while the "irrigation and swish" technique demonstrates a predominance of Gram-negative and equal anaerobic and facultative anaerobes. The micropipette analysis of the periodontal disease pockets demonstrates a predominance of Gram-negative and anaerobic bacteria in diseased pockets that increases as the pocket depth increases. Different evaluation methods of the same patients should coincide, not diverge. If the micropipette analysis is the most accurate, the "irrigation and swish" is the next most accurate and the most inaccurate is the "swish" analysis. This study is a small sample and these results should be evaluated in larger studies with a greater in-depth analysis.

CONCLUSION

This research helps clarify the biofilm found in healthy periodontal tissues, which varies significantly from the biofilm found in diseased tissues. It is important to know what biofilm constituents exist in the host tissues to determine health or disease as this may be important in determining treatment success or failure. The three methods of evaluating the biofilm, "swish", "irrigation and swish" and micropipette analysis coincide with regard to the biofilm in healthy tissues, but the results vary and are uncertain when evaluating the etiology of disease.

Three methods of analysis are compared in this study and all three methods ("swish", "irrigation and swish" and micropipette) demonstrate similar findings with healthy periodontal tissue. The three analyzes for healthy periodontal tissues (3 mm or less with no BOP) demonstrates Gram-positive bacteria and facultative anaerobes predominate. There are lesser amounts of anaerobic bacteria, aerobic bacteria and fungi. All three analysis methods generally agree with regard to healthy tissues, but this similarity is missing with regard to diseased tissues.

The three methods evaluate the type of bacteria; Gram-positive or Gram-negative. The "swish" analysis for patients with periodontal disease demonstrates a predominance of Gram-positive bacteria. The "irrigation and swish" analysis of patients with periodontal disease and the micropipette analysis of periodontal pockets 4 mm or greater demonstrate a predominance of Gram-negative bacteria. The micropipette analysis demonstrates Gram-negative bacterial predominance increases as pocket depth increases.

The categories of bacteria vary in the analysis of periodontal disease patients between the "swish", "irrigation and swish" and the micropipette techniques. The "swish" analysis of periodontal disease patients demonstrates facultative anaerobes predominate, followed by anaerobes, aerobic bacteria and fungi. The "irrigation and swish" analysis of periodontal disease patients demonstrates a comparable facultative and anaerobic population with a lesser presence of aerobic bacteria and fungi. The micropipette analysis demonstrates anaerobic bacteria predominate at 3 mm in periodontal disease patients and the predominance increases as the pocket depth increases.

The variability of the findings is troubling since the tests were completed on the same patients. One would expect the findings to coincide, but this is not what occurred. Comparison of the oral saliva "swish" test with the "irrigation and swish" and the micropipette analysis of the type and category of bacteria demonstrates significant imprecisions. If the micropipette analysis best represents the microbiome in the periodontal pocket, the "irrigation and swish" analysis is less accurate and the "swish" analysis provides the most inaccurate information.

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