Article:
Baraniya, D, Naginyte, M, Chen, T et al. (5 more authors) (2020) Modeling Normal and Dysbiotic Subgingival Microbiomes: Effect of Nutrients. Journal of Dental Research, 99 (6). pp. 695-702. ISSN 0022-0345

https://doi.org/10.1177/0022034520902452

Modeling normal and dysbiotic subgingival microbiomes: effect of nutrients

Divyashri Baraniya[1], Monika Naginyte[2], Tsute Chen[3], Jasim M. Albandar[4], Susan M. Chialastri[4], Deirdre A. Devine[2], Philip D. Marsh[2] and Nezar Noor Al-hebshi [1*]

[1] Oral Microbiome Research Laboratory, Maurice H. Kornberg School of Dentistry, Temple University, Philadelphia, USA

[2] Division of Oral Biology, School of Dentistry, University of Leeds, Leeds, UK

[3] Department of Microbiology, Forsyth Institute, Cambridge, MA, USA

[4] Department of Periodontology and Oral Implantology, Maurice H. Kornberg School of Dentistry, Temple University, Philadelphia, USA

[*]**Corresponding author**

Nezar Noor Al-hebshi. Maurice H. Kornberg School of Dentistry, Temple University, Philadelphia, USA. Tel: +12157072091. E-mail address: alhebshi@temple.edu

Running title: Replicating subgingival normobiosis and dysbiosis

Abstract

Screening for microbiome modulators requires availability of a high throughput in vitro model that replicates subgingival dysbiosis and normobiosis, along with a tool to measure microbial dysbiosis. Here, we tested various formulations to grow health- and periodontitis-associated subgingival microbiomes in parallel, and describe a new subgingival dysbiosis index. Subgingival plaque samples pooled from five healthy, and separately, five periodontitis subjects were used to inoculate a Calgary Biofilm device containing saliva-conditioned, hydroxyapatite-coated pegs. Microbiomes were grown for 7 days on either nutrient-rich media, including a modification of SHI (mSHI), BHI supplemented with hemin and vitamin K (sBHI), and a blend of SHI and BHI (BSHI), each at three sucrose concentrations (0%, 0.05% and 0.1%), or nutrient-limited media (saliva with 5%, 10% or 20% inactivated human serum). The microbiomes were assessed for biomass, viability, and 16S rRNA profiles. In addition to richness and diversity, a dysbiosis index was calculated as the ratio of the sum of relative abundances of disease-associated species to that of health-associated species. sBHI and BSHI resulted in the highest biomass, whereas saliva-serum maximized viability. Distinct groups of bacteria were enriched in the different media. Regardless of medium type, the periodontitis-derived microbiomes showed higher species richness and alpha diversity and clustered with the respective inoculum separately from the health-derived microbiomes. Microbiomes grown in saliva-serum showed the highest species richness, and the highest similarity to the clinical inocula, both in health and disease. However, inclusion of serum reduced alpha diversity and increased dysbiosis in healthy microbiomes in a dose-dependent manner, mainly due to over-enrichment of Porphyromonas species. mSHI, stood second in terms of species richness and diversity, but resulted in low biomass and viability, and significantly worsened dysbiosis in the periodontitis-derived microbiomes. Overall, saliva with 5% human serum was optimal for replicating subgingival microbiomes from health and disease.

Key words: Biofilm; Dysbiosis; High-Throughput Nucleotide Sequencing; Microbiota; Periodontitis

Introduction

An emerging strategy for prevention and adjunctive treatment of periodontitis is to selectively target keystone pathogens or/and stimulate growth of commensals to reverse microbial dysbiosis (or re-establish normobiosis) by using microbiome modulators, such as prebiotics or probiotics. Both prebiotics and probiotics have been extensively studied within the context of gut heath (Gareau et al. 2010; Holscher 2017); however, their applicability, particularly prebiotics, to periodontal health – and oral health generally – has been minimally explored. A major obstacle has been the lack of a reliable in vitro tool for the screening of banks of potential modulators to identify those with promising activities before testing them further in animals, and eventually, humans. Recently, Slomka et al. (Slomka et al. 2017) used a dual-species biofilm model to screen a panel of 704 nutritional compounds against 16 oral bacterial species for prebiotic activity. However, dual-species biofilms are far from being representative of complex oral microbial communities.

Classically, dental biofilm models for the screening of antibacterials or mouthwashes have included a limited number of oral species (Ammann et al. 2013; Guggenheim et al. 2001), while more complex biofilms have been produced from clinical inocula in constant depth fermenters or CDC biofilm reactors (Hope and Wilson 2006; Rudney et al. 2012). Although the latter systems replicate the dynamic conditions of the mouth, they are complex and costly, have low throughput and are more suited for studying biofilm development and structure (Darrene and Cecile 2016). More recently, oral microbiome models have been successfully generated from pooled saliva samples in cheaper, high-throughput devices, such as microtiter plates or the Calgary Biofilm Device (CBD) (Edlund et al. 2013; Kistler et al. 2015; Kolderman et al. 2015; Tian et al. 2010). While these models are static, the biofilms generated capture a great deal of the species and functional diversity of the original samples. Furthermore, when subgingival plaque samples from healthy and periodontitis patients were used as inocula, biofilms were generated with a close similarity to the clinical inocula with clear distinction between the health- and periodontitis-derived microbiomes

(Velsko and Shaddox 2018; Walker and Sedlacek 2007). However, none of these models have been designed for studying microbiome modulation.

The current study is one in a series aimed to establish a high throughput, reproducible in vitro subgingival microbiome model, specifically optimized for the testing of microbiome modulators. Here, the objective was to identify the optimal medium for replicating subgingival normobiosis and dysbiosis in vitro, and to describe a new subgingival dysbiosis index that can be used to quantitatively assess dysbiosis and microbiome modulation.

Materials and methods

Clinical inocula and saliva

Subgingival dental plaque samples were collected from five patients with untreated, moderate to severe periodontitis (defined as having at least one tooth per quadrant with bleeding on probing, pocket depth ≥ 5mm and attachment loss ≥ 4 mm) and five periodontally-healthy controls (defined as having no more than slight gingivitis and no probing pocket depth or attachment loss ≥ 3mm) with no previous history of periodontitis. Subgingival plaque was sampled by inserting a size 40 paper point to the base of gingival sulcus/ pocket for 30s. Samples were obtained from the deepest pocket in each quadrant in the periodontitis patients, and from the buccal gingival sulcus of first molars in the healthy subjects. The samples from each subject were pooled in 1 ml reduced transport fluid (Hoover and Newbrun 1977) and placed on ice for use on the same day.

Separately, unstimulated saliva samples (5-10 ml) were collected from each of 10 dentally-healthy volunteers (distinct from the 5 healthy controls described above) and centrifuged at 5000 rpm for 15 minutes. The supernatants were pooled, treated with dithiothreitol (2.5 mM final concentration) for 10 minutes, mixed with equal volume of phosphate buffered saline (PBS), filter-sterilized and stored at -20°C.

The study was approved by the Temple University's Institutional Review Board (protocol # 25586).

Growth media

Nutrient-rich and nutrient-limited media were used to grow the microbiomes. The former included Brain Heart Infusion broth (Difco, Becton Dickinson, USA) supplemented with hemin (5 mgL^{-1}), vitamin K (0.5 mgL^{-1}) and mucin (1 mgL^{-1}) (sBHI medium); a modification of SHI medium (Tian et al. 2010) in which potassium chloride was replaced by PBS for buffering (mSHI medium); and a blend of mSHI and sBHI (BSHI hereafter), prepared by adding together the non-redundant components from both media. Each medium was tested at three sucrose concentrations (0%, 0.05% and 0.1% w/v). The nutrient-limited media comprised sterile saliva (prepared as described above) containing 5%, 10% or 20% (v/v) heat-inactivated serum (Sigma Aldrich, USA). In all, a total of 12 media were compared (find detailed composition in **Appendix Tables 1 and 2**).

Growing the microbiomes

Subgingival plaque samples were briefly vortexed and pooled, separately for the periodontitis patients and healthy controls, to make two inocula. Periodontitis- and health-derived microbiomes were grown in triplicate in each medium on a Calgary Biofilm Device (Ceri et al. 1999) with hydroxyapatite-coated pegs (CBD; Innovotech, Edmonton, Canada), at 37°C in an anaerobic chamber (10% hydrogen, 10% carbon dioxide, and 80% nitrogen). The plate layout is shown in **Appendix Figure 1**. Outer wells were not used to prevent evaporation. The different microbiomes were separated from each other and from the negative control by empty wells filled with 180 µL PBS to avoid well-to-well contamination. The pegs were preconditioned by immersion in sterile human saliva for 16 hours prior to inoculation. Experimental wells contained 170 µL growth medium and 10 µl of the respective pooled clinical sample (sterile PBS for negative control wells). The plate was incubated for 7 days with media replenished on days 2, 4 and

6. Microbiomes (for each inoculum and medium type) were generated in two sets; one was used immediately for measurement of viability and the other for extraction of DNA. A portion of each pooled clinical sample was kept aside for microbiome analysis.

Measurement of biomass and viability

The pegs with microbiomes were washed three times with PBS to remove planktonic bacteria. Biomass was measured in terms of DNA yield (ng per microbiome). For DNA extraction, pegs were snapped off and each was placed in an Eppendorf tube with 180 µl PBS containing 18 µl MetaPolyzyme (Sigma, USA) and incubated at 35°C for 4 hours. The digests were then used for DNA extraction using the Purelink Genomic Kit (Life Technologies, USA), following the manufacturer's instructions. DNA from the clinical samples was extracted similarly. To account for extracellular DNA (eDNA) possibly present in saliva (Okshevsky and Meyer 2015), a saliva-serum medium-only control was included. DNA was quantified by Qubit ® 2.0 Fluorimeter (Life Technologies, USA) before storing at -80°C.

Viability of the microbiomes was directly assessed on the pegs (i.e. without harvesting the bacteria) using an ATP assay (BacTiter-Glo assay, Promega, USA) according to the manufacturer's modified protocol for biofilms (https://www.promega.com/-/media/files/resources/promega-notes/99/use-of-the-bactiter-glo-microbial-cell-viability-assay-to-study-bacterial-attachment.pdf?la=en). Luminescence signal was recorded on a Synergy HTX multi-mode microplate reader (Biotek, USA) and normalized to biomass.

16S sequencing and bioinformatic analysis

16S rRNA gene library preparation and sequencing were performed at the Australian Center for Ecogenomics (Brisbane, Australia) as described previously (Al-Hebshi et al. 2017a). Briefly, the degenerate primers 27FYM (Frank et al. 2008) and 519R (Lane et al. 1985) were used to amplify the V1-

3 region using standard PCR conditions. The resultant PCR amplicons (~ 520 bp) were purified, indexed with unique 8-base barcodes in a second PCR, pooled together in equimolar concentrations and sequenced employing the v3 2x300 bp chemistry on a MiSeq platform (Illumina, USA) at 30,000 reads/sample. No detectable eDNA was found in the saliva-serum medium-only control (using the Qubit dsDNA High Sensitivity kit), and so it was not sequenced.

Preprocessing of data (primer trimming, merging of reads, quality-filtration, alignment and chimera removal) was done as described previously (Al-Hebshi et al. 2017b). The high quality, merged reads were classified to the species level using our BLASTn-based algorithm (Al-Hebshi et al. 2015; Al-Hebshi et al. 2017b). The QIIME (Quantitative Insights Into Microbial Ecology) software package version 1.9.1 (Caporaso et al. 2010) integrated into our analysis pipeline was used for downstream analysis including subsampling, generation of taxonomy plots/tables and rarefaction curves, and calculation of species richness, coverage, alpha diversity indices and beta diversity distance matrices. Principal component analysis (PCoA) was used to visualize the distances between the microbiomes. Linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al. 2011) was used to detect taxa enriched by the different media, adjusting for multiple comparison with the Benjamini-Hochberg method. To quantitatively assess the similarity of the generated microbiomes to the clinical inocula, a similarity index was calculated as 1-abundance-weighted Jaccard distance from the respective clinical inoculum.

Subgingival dysbiosis index

A subgingival dysbiosis index (SDI), inspired from the dysbiosis index described by Greves et. al. for Crohn's disease (Gevers et al. 2014) was calculated for each generated microbiome as follows:

$$SDI = \frac{\sum relative\ abundances\ of\ periodontitis\ associated\ species}{\sum relative\ abundances\ of\ health\ associated\ species}$$

where periodontitis-associated species are all of the species that were more abundant in the periodontitis clinical inoculum compared with the healthy clinical inoculum, and vice versa (**Appendix Dataset 1**)

Results

Raw data are available from Sequence Read Archive (PRJNA579567). Summary and detailed sequencing and data preprocessing statistics are provided in **Appendix materials (Appendix Dataset 2).**

Biomass and viability

The biomass and viability of the microbiomes generated in the different media are shown in **Figure 1**. sBHI and BSHI, resulted in the highest biomass, with the periodontitis-derived microbiomes having a significantly higher biomass than the health-derived ones, regardless of sucrose concentration (1712±416 ng vs. 1055±389 ng for BSHI; 1438±113 ng vs. 771±128 ng for sBHI). However, the viability of periodontitis-derived microbiomes was very low in both media. The microbiomes grown in mSHI had the lowest biomass (144±74 ng and 421±185 ng for the disease- and health-derived microbiomes, respectively), and both also displayed low viability. The addition of sucrose to the three media did not have a consistent effect. For example, including sucrose at 0.1% in sBHI and BSHI significantly enhanced viability of the health-derived microbiomes, but not the periodontitis-associated microbiomes; it also reduced biomass of both types of microbiomes grown in BSHI, but only health-associate microbiomes grown in sBHI, while it tended to increase it in mSHI-grown microbiomes. The saliva-serum media generated microbiomes with intermediate biomass (461±286 ng and 453±145 ng for the disease- and health -derived microbiomes, respectively), but also the highest viability, especially in the periodontitis-derived microbiomes (on average, ~4-10 times higher compared to other media). Increasing the serum concentration to 10% (v/v) improved biomass and viability of the microbiomes; at 20% (v/v) serum, biomass further increased but viability was adversely affected.

Microbiological profiles by general medium type

All phyla, 71 out of 74 genera and 224 out of 231 species present in the clinical inocula were also detected in at least one of the in vitro microbiome subgroups (**Appendix Datasets 2-4**). The average relative abundances of phyla and top genera (accounting for ~ 80% of the sequences) in the microbiomes generated and the respective clinical inocula are presented in **Figure 2**. Major phyla identified in both the inocula and grown microbiomes were Firmicutes, Fusobacteria and Bacteroidetes. All media significantly enriched for Firmicutes, while hardly supported the growth of Proteobacteria. At the genus level, Haemophilus, Leptotrichia, Aggregatibacter, Capnocytophaga, Fretibacterium and Mycoplasma were among the major genera present in the clinical inocula but were found in low abundance (or absent) in the in vitro microbiomes. Conversely, genera Parvimonas, Mogibacterium, Oribacterium, Atopobium, Dialister, Eggerthia and Peptostreptococcus, were over-represented in the microbiomes compared to the respective inocula, irrespective of the medium used. Interestingly, there were 25 species (6 genera) including Mogibacterium neglectum, Mogibacterium pumilum, Alloscardovia omnicolens, Acidaminococcus sp. str. D21 and several potentially novel OTUs that were not detected in the clinical inocula, but were present in the derived microbiomes (**Appendix Dataset 5**). The microbial profiles were highly reproducible between the replicates (**Appendix Figure 2**)

Different bacteria were enriched in the various media as revealed by LEfSe analysis (**Appendix Figure 3**). Saliva-serum enriched for Bacteriodetes (genera Porphyromonas and Alloprevotella) and Spirochaetes (Genus Treponema); sBHI enriched for Firmicutes (genera Veillonella and Peptostreptococcus) and Saccharibacteria; mSHI enhanced the growth of genera Fusobacterium, Streptococcus and Tannerella; while BSHI favored the genus Prevotella. The most pronounced enrichment was that of Porphyromonas gingivalis in the health-derived microbiomes grown in saliva-serum (relative abundance of ~30% compared to 5.8% in the respective clinical inoculum and less than 0.1% in microbiomes grown in other media). Similarly, Pyramidobacter piscolens was significantly

enriched in the periodontitis-derived microbiomes grown in sBHI, reaching a relative abundance of 11.0% compared with 1.7% in the periodontitis inoculum.

Species richness, alpha- and beta-diversity

The species richness and alpha-diversity indices are presented in **Figure 3**. Saliva-serum was associated with the highest observed and expected (Chao) species richness, especially in the periodontitis-derived microbiomes, supporting growth of up to 160 species from the clinical inocula; however, a higher serum concentration (especially at 20%) was associated with lower species richness. mSHI generated a comparable richness in the health-derived microbiomes to that of microbiomes grown in saliva-serum (125-140 observed species). Growth of microbiomes in saliva-serum, however, resulted in a significant drop in Shannon's and Simpson's indices, particularly in the health-derived microbiomes in which the reduction was serum concentration-dependent. Overall, BSHI resulted in the lowest species richness and alpha-diversity. Including sucrose in BSHI, mSHI or sBHI increased species richness, but did not have a consistent effect on alpha-diversity.

The results of clustering of the microbiomes and clinical inocula by PCoA are shown in **Figure 4 A and B**. Regardless of the medium used, the periodontitis-derived microbiomes clustered with the periodontitis inoculum separately from the health-derived microbiomes, accounting for the variation along principle coordinate (PC) 1 (44.55%). Differences by medium type accounted for variation along PC2 (31.15%). The microbiomes grown in saliva-serum clustered closest to the clinical inocula followed by those generated in mSHI. To better visualize the similarity of the microbiomes to their respective clinical inocula by specific medium type, the similarity index was calculated and plotted as presented in **Figure 4C**. In both health- and periodontitis-derived microbiomes, saliva with 5% serum resulted in the highest similarity to the clinical inocula. Increasing serum concentration reduced similarity, but the microbiomes generated still had a greater similarity than those grown in mSHI. Including sucrose in

mSHI enhanced similarity, but only for the health-derived microbiomes. BSHI and sBHI generated microbiomes with the least similarity to the clinical inocula.

Dysbiosis

All media replicated the normobiotic and dysbiotic states of the respective clinical inocula to some degree (**Figure 5**), although there were significant differences among them. sBHI and BSHI media generated health-derived microbiomes with an SDI very close to that of the respective clinical sample, but were associated with a significant drop in dysbiosis of the periodontitis-derived microbiomes, resulting in a difference in SDI of 0.9 to 1.4 between the normobiotic and corresponding dysbiotic microbiomes (compared to a difference of 1.84 between the clinical inocula). Growth in mSHI resulted in an extreme difference in SDI of 2.4-3.2 by lowering dysbiosis in the health-derived microbiomes relative to the healthy clinical inoculum and significantly worsening it in the periodontitis-derived microbiomes. Saliva with 5% serum generated periodontitis-derived microbiomes with the closest SDI to that of the periodontitis sample, but worsened dysbiosis in the health-derived microbiomes; nevertheless, it nearly replicated the difference in SDI between the clinical inocula (1.80 vs. 1.84). Higher serum concentrations increased dysbiosis in both microbiome types and resulted in an SDI difference of 2.2 and 1.6 for 10% and 20% serum, respectively.

Discussion

In this study, we tested various media to replicate health- and periodontitis-associated subgingival microbiomes. BHI supplemented with hemin, mucin and vitamin K has been shown to support growth of a diverse microbial community, including periodontal pathogens, from saliva inocula (Kistler et al. 2015). The original SHI with 0.5% sucrose has been primarily used to replicate microbiomes associated with dental caries (Edlund et al. 2013); however, lowering the sucrose concentration (0.1%) had resulted

in higher proportions of subgingival species, which is why we evaluated it and compared different sucrose concentrations. The combined medium (BSHI) was developed based on the assumption that it could maximize the number of species in the microbiomes. Saliva-serum was included as a nutrient-limited medium that simulates nutritional conditions in the gingival crevice/pocket. More details about the choice of methods and their limitations are provided in the **Appendix Discussion.**

Regardless of the medium, the health- and periodontitis-derived microbiomes clustered separately, consistent with previous work (Fernandez et al. 2017; Velsko and Shaddox 2018), indicating that the final composition of the generated microbiomes is largely dictated by that of the respective inocula. All media also replicated normobiosis and dysbiosis to some degree, demonstrating the overall validity of the model. Nevertheless, there were differences between the media worth highlighting, such as the relationship between biomass and viability. sBHI and BSHI resulted in high biomass but low viability suggesting that they provide an early boost to the growth of bacteria such that a significant proportion of the microbiome enters the stationary and decline phases of growth before the medium is replenished. This may also explain the lower species richness observed in these media, as slowly growing species were probably outcompeted. Perhaps, growth rates in the less rich saliva media were lower and population growth was more balanced resulting in a better relationship between final biomass and viability. This might explain why saliva-serum resulted in the highest species richness, and hence, greater similarity to the clinical inocula. mSHI was an outlier in that it resulted in both low biomass and viability especially in the periodontitis-derived microbiomes, which warrants further investigation.

The dysbiosis index provided a valuable layer of information in addition to the standard microbiome metrics, and reflected another important difference between the tested media. Compared with the clinical inocula, sBHI and BSHI narrowed the difference in SDI between the health- and periodontitis-derived microbiome, while mSHI widened it. On the other hand, saliva-serum (especially at 5% serum) nearly replicated the difference between the clinical inocula, despite over-enrichment of P. gingivalis in the health-derived microbiomes, indicating that normobiosis/dysbiosis is a microbial

community feature not dependent on a single species. In other words, the high abundance of P. gingivalis was accompanied by decreases in the abundance of other periodontitis-associated species and maintenance of commensal/health-associated species, hence the low SDI and a difference between healthy and diseased microbiomes similar to that seen comparing the inocula. Nevertheless, over-enrichment of P. gingivalis remains noteworthy since we cannot exclude if such a microbiome (high P. gingivalis/low SDI) would still be pathogenic. Since serum is probably enriching for P. gingivalis (Cieplik et al. 2019; Naginyte et al. 2019), lowering its concentration may overcome this limitation. Another caveat worth mentioning, is that the SDI was calculated based on the differences between the clinical inocula, which limits its validity to the experimental run. We are currently developing a more generic index based on the relative abundances of a predefined set of health and periodontitis-associated species, that can be compared across experimental runs and even for assessment of clinical samples.

In conclusion, we describe here a model system, and a novel dysbiosis index, that could form the basis of a high throughput model for screening microbiome modulators. Overall, saliva-serum is probably the optimal medium for modelling the subgingival microbiome by maximizing species diversity and maintaining viability, while replicating normobiosis/dysbiosis.

Author contributions

D. Baraniya and M. Naginyte contributed to design, acquisition and analysis of the data, and critically revised the manuscript. T. Chen contributed to analysis of the data and critically revised the manuscript. J. M. Albandar and S. M. Chialastri contributed to acquisition of the data and critically revised the manuscript. P. D. Marsh and D. A. Devine contributed to design, interpretation of the data and critically revised the manuscript. N. Al-hebshi contributed to conception and design, data analysis and interpretation and drafted and critically revised the manuscript. All co-authors gave final approval and

agree to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgements

This study was supported by the National Institute of Dental and Carniofacial Research (Grant 1R03DE028379-01A1). There are no conflicts of interest to disclose.

References

Al-Hebshi NN, Alharbi FA, Mahri M, Chen T. 2017a. Differences in the bacteriome of smokeless tobacco products with different oral carcinogenicity: Compositional and predicted functional analysis. Genes.in press.

Al-Hebshi NN, Nasher AT, Idris AM, Chen T. 2015. Robust species taxonomy assignment algorithm for 16s rrna ngs reads: Application to oral carcinoma samples. J Oral Microbiol. 7:28934.

Al-Hebshi NN, Nasher AT, Maryoud MY, Homeida HE, Chen T, Idris AM, Johnson NW. 2017b. Inflammatory bacteriome featuring fusobacterium nucleatum and pseudomonas aeruginosa identified in association with oral squamous cell carcinoma. Sci Rep. 7(1):1834.

Ammann TW, Bostanci N, Belibasakis GN, Thurnheer T. 2013. Validation of a quantitative real-time pcr assay and comparison with fluorescence microscopy and selective agar plate counting for species-specific quantification of an in vitro subgingival biofilm model. J Periodontal Res. 48(4):517-526.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI et al. 2010. Qiime allows analysis of high-throughput community sequencing data. Nature methods. 7(5):335-336.

Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. 1999. The calgary biofilm device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. Journal of Clinical Microbiology. 37(6):1771-1776.

Cieplik F, Zaura E, Brandt BW, Buijs MJ, Buchalla W, Crielaard W, Laine ML, Deng DM, Exterkate RAM. 2019. Microcosm biofilms cultured from different oral niches in periodontitis patients. J Oral Microbiol. 11(1):1551596.

Darrene LN, Cecile B. 2016. Experimental models of oral biofilms developed on inert substrates: A review of the literature. Biomed Res Int. 2016:7461047.

Edlund A, Yang Y, Hall AP, Guo L, Lux R, He X, Nelson KE, Nealson KH, Yooseph S, Shi W et al. 2013. An in vitro biofilm model system maintaining a highly reproducible species and metabolic diversity approaching that of the human oral microbiome. Microbiome. 1(1):25.

Fernandez YMM, Exterkate RAM, Buijs MJ, Beertsen W, van der Weijden GA, Zaura E, Crielaard W. 2017. A reproducible microcosm biofilm model of subgingival microbial communities. J Periodontal Res. 52(6):1021-1031.

Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. 2008. Critical evaluation of two primers commonly used for amplification of bacterial 16s rrna genes. Appl Environ Microbiol. 74(8):2461-2470.

Gareau MG, Sherman PM, Walker WA. 2010. Probiotics and the gut microbiota in intestinal health and disease. Nat Rev Gastroenterol Hepatol. 7(9):503-514.

Gevers D, Kugathasan S, Denson LA, Vazquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Song SJ, Yassour M et al. 2014. The treatment-naive microbiome in new-onset crohn's disease. Cell Host Microbe. 15(3):382-392.

Guggenheim B, Giertsen E, Schupbach P, Shapiro S. 2001. Validation of an in vitro biofilm model of supragingival plaque. J Dent Res. 80(1):363-370.

Holscher HD. 2017. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes. 8(2):172-184.

Hoover CI, Newbrun E. 1977. Survival of bacteria from human dental plaque under various transport conditions. J Clin Microbiol. 6(3):212-218.

Hope CK, Wilson M. 2006. Biofilm structure and cell vitality in a laboratory model of subgingival plaque. J Microbiol Methods. 66(3):390-398.

Kistler JO, Pesaro M, Wade WG. 2015. Development and pyrosequencing analysis of an in-vitro oral biofilm model. BMC Microbiol. 15:24.

Kolderman E, Bettampadi D, Samarian D, Dowd SE, Foxman B, Jakubovics NS, Rickard AH. 2015. L-arginine destabilizes oral multi-species biofilm communities developed in human saliva. PLoS One. 10(5):e0121835.

Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR. 1985. Rapid determination of 16s ribosomal rna sequences for phylogenetic analyses. Proc Natl Acad Sci U S A. 82(20):6955-6959.

Naginyte M, Do T, Meade J, Devine DA, Marsh PD. 2019. Enrichment of periodontal pathogens from the biofilms of healthy adults. Sci Rep. 9(1):5491.

Okshevsky M, Meyer RL. 2015. The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms. Crit Rev Microbiol. 41(3):341-352.

Rudney JD, Chen R, Lenton P, Li J, Li Y, Jones RS, Reilly C, Fok AS, Aparicio C. 2012. A reproducible oral microcosm biofilm model for testing dental materials. J Appl Microbiol. 113(6):1540-1553.

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12(6).

Slomka V, Hernandez-Sanabria E, Herrero ER, Zaidel L, Bernaerts K, Boon N, Quirynen M, Teughels W. 2017. Nutritional stimulation of commensal oral bacteria suppresses pathogens: The prebiotic concept. J Clin Periodontol. 44(4):344-352.

Tian Y, He X, Torralba M, Yooseph S, Nelson KE, Lux R, McLean JS, Yu G, Shi W. 2010. Using dgge profiling to develop a novel culture medium suitable for oral microbial communities. Mol Oral Microbiol. 25(5):357-367.

Velsko IM, Shaddox LM. 2018. Consistent and reproducible long-term in vitro growth of health and disease-associated oral subgingival biofilms. BMC Microbiol. 18(1):70.

Walker C, Sedlacek MJ. 2007. An in vitro biofilm model of subgingival plaque. Oral Microbiol Immunol. 22(3):152-161.

Figure legends

Figure 1. Biomass and viability of the microbiomes grown in the different media. Biomass was measured as the yield of DNA in nanograms extracted from the microbiomes. Viability was assessed using a luminescence ATP assay, normalizing the relative luminescence signal to biomass.

Figure 2. Microbiological profiles. The relative abundances of phyla (upper panel) and major genera (lower panel) identified in the clinical inocula and respective microbiomes grown in the different media (data presented for the four general media types).

Figure 3. Species richness and alpha diversity. Taxonomic profiles were rarified and used to calculate observed richness, expected richness (Chao index), and alpha diversity indices (Shannon's and Simpson's) for each of the clinical inocula and respective microbiomes grown in the different media, employing standard QIIME scripts.

Figure 4. Beta diversity analysis. Distances between the microbiomes were calculated based on abundance-weighted Jaccard index employing standard QIIME scripts. The microbiomes were clustered using Principle Coordinate Analysis (PCoA) by **A**) the respective clinical inoculum and **B**) general medium type. The clinical inocula are represented by rhomboid icons. C) The similarity of the microbiomes to the clinical inocula from which they were grown, calculated for each microbiome as 1-abundance-weighted Jaccard distance from the respective clinical inoculum. PC: principle coordinate.

Figure 5. The level of dysbiosis in the clinical inocula and respective microbiomes grown in the different media as assessed by a subgingival dysbiosis index (SDI) calculated as [total abundance of all species increased in the periodontitis inoculum] over [total abundance of all species increased in the healthy inoculum]. Higher values indicate a greater level of dysbiosis.