Salivary protein glycopatterns for natural regulation of oral microbiota

Zheng Li (zhengli@nwu.edu.cn)
Northwest University https://orcid.org/0000-0002-1839-3450
Jian Shu
Northwest University
Yu Hanjie
Northwest University
Yan Wang
Northwest University
Xiameng Ren
Northwest University
Kun Zhang
Northwest University
Zhen Tang
Northwest University
Wentian Chen
Northwest University
Baozhen Li
The First Affiliated Hospital of Xi'an Jiaotong University

Biological Sciences - Article

Keywords: oral microbiota, glycoconjugates, oral health

Posted Date: February 12th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-221122/v1

Abstract

The oral microbiota is the direct precursor of dental caries and periodontitis, which are the most common microbial-induced diseases worldwide. The distinct microenvironment at the oral barrier breeds unique microbial communities, which are regulated by host factors (inflammation or dietary sugars)1. Increasing evidence indicates that dysbiosis of oral microbial communities is associated with many human diseases2-5. Our studies demonstrated that human disease could induce different alterations in salivary protein glycopatterns6. However, the relationship between salivary protein glycopatterns and oral microbial communities is unknown. Here, we report that altered salivary protein glycopatterns, namely, fucosylated or sialylated structures induced by gastric cancer (GC) or type 2 diabetes mellitus (T2DM), respectively, are also drivers of dysbiosis of oral microbial communities and ultimately dental caries and periodontitis. The fucosylated neoglycoproteins and sialic acid (SA) α2-3 galactose (Gal) structure can inhibit the growth or/and adhesion of Aggregatibacter segnis and Candida albicans from the oral cavity of patients with GC and T2DM, respectively. These findings provide a novel theory that dynamic communities of oral microbiota are regulated naturally by host salivary protein glycopatterns, having important implications for developing new carbohydrate drugs for oral and body health.

Main Text

The microbiota in the oral cavity plays an important role in maintaining oral health and even body health. The nososymbiocity of the polymicrobial communities involved in both dental caries and periodontitis is largely regulated by host factors, predominantly dietary sugars and inflammation. Recently, increasing evidence has shown that the oral microbiota is associated with human diseases, including diabetes, obesity, and cancer[7,8]. Our previous studies demonstrated that different types of diseases (e.g., gastric cancer (GC), type 2 diabetes mellitus (T2DM), hepatopathy, and breast disease) could induce various alterations in salivary protein glycopatterns (e.g., fucosylation in GC and sialylation in T2DM)[9-12]. Furthermore, can altered salivary protein glycopatterns also impact the dynamic and polymicrobial oral microbiome? We hypothesize that altered salivary protein glycopatterns are possible drivers leading to dysbiosis of oral microbial communities and ultimately dental caries and periodontitis.

To address this hypothesis, we first studied the changes in the oral microbiota associated with GC. Among the 24 samples, an estimated 79 thousand raw sequence reads per sample with a median length of 423 base pairs is obtained. After quality trimming and chimera checking, 75 thousand clean sequence reads per sample remained, which are recovered for downstream analysis. The detailed sequencing data of each sample are shown in Extended Data Table S1. In total, 789 unique species are identified in the oral cavity of healthy volunteers (HVs) and patients with GC; 525 species are common in the HVs and GC patients, while 128 and 136 species are observed in only the HVs and GC patients, respectively (Fig. 1A). The alterations in the microbial communities are evaluated between the HV and GC groups. The alpha-diversity analysis shows that there are no significant differences between the groups (Fig. 1B-E), while the beta-diversity analysis shows that the oral microbiota profiles of the two groups are different, and can be distinguished using PCA, PCoA and NMDS plots (Fig. 1F-H).

To further investigate the composition of oral microbial communities, the Wilcoxon test is used to compare the variance at each taxonomic level between the HV and GC groups (Table 1). The results show that there are no significant differences at the phylum, class and order levels; however, the members of Carnobacteriaceae are increased in the GC patients compared with the HVs at the family level (Fig. 2A). The proportions of the genera *Alloprevotella* and *Megasphaera* are significantly increased whereas those of *Granulicatella*, *Bregeyella* and *TM7[G-6]* are decreased in the GC group (Fig. 2B). Five species (*Oral taxon 392, Oral taxon 308*, *Aggregatibacter segnis (A. segnis)*, *Megasphaera micronuciformis (M. micronuciformis),* and *Oral taxon 396*) are significantly increased ($p<0.05$) and 2 (*Streptococcus salivarius (S. salivarius)* and *Oral taxon 870*) are significantly decreased ($p<0.01$) in the GC patients compared with the HVs (Fig. 2C). Furthermore, the relative abundances of *A. segnis, M. micronuciformis* and *S. salivarius* are verified by PCR and qPCR, and their results are consistent with the results obtained by 16S rDNA sequencing (Extended Data Fig. S1).

Table 1 Taxonomic differences in the oral microbiota of HV and GC.

Taxa (class; order; family; genus; species)	HV		GC		*p*
	Avg, % (Carriage, %)	SD	Avg, % (Carriage, %)	SD	
Phylum _ Bacteroidetes					
c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Alloprevotella	0.84 (100)	0.81	1.85 (100)	1.28	0.032
c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Alloprevotella; s__sp._oral_taxon_308	0.27 (100)	0.16	0.66 (100)	0.48	0.019
c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__sp._oral_taxon_396	0.05 (92)	0.04	0.12 (100)	0.11	0.046
c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__Bergeyella	0.42 (100)	0.47	0.11 (100)	0.10	0.047
Phylum _ Firmicutes;					
c__Bacilli; o__Lactobacillales; f__Carnobacteriaceae	2.89 (100)	2.24	1.39 (100)	0.85	0.048
c__Bacilli; o__Lactobacillales; f__Carnobacteriaceae; g__Granulicatella	2.89 (100)	2.24	1.39 (100)	0.85	0.048
c__Negativicutes; o__Selenomonadales; f__Veillonellaceae; g__Megasphaera	0.03 (75)	0.04	0.15 (100)	0.17	0.033
c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__salivarius	**0.27 (100)**	0.21	**0.07 (100)**	0.08	0.007
c__Negativicutes; o__Selenomonadales; f__Veillonellaceae; g__Megasphaera; s__micronuciformis	**0.03 (75)**	0.04	**0.15 (100)**	0.17	0.036
Phylum _ Fusobacteria;					
c__Fusobacteriia; o__Fusobacteriales; f__Leptotrichiaceae; g__Leptotrichia; s__sp._oral_taxon_392	0.46 (100)	0.30	1.02 (100)	0.74	0.029
Phylum _ Proteobacteria					
c__Gammaproteobacteria; o__Pasteurellales; f__Pasteurellaceae; g__Aggregatibacter; s__segnis	**0.10 (100)**	0.14	**0.41 (75)**	0.44	0.032
Phylum _ Saccharibacteria_(TM7)					
c__TM7_[C-1]; o__TM7_[O-1]; f__TM7_[F-1]; g__TM7_[G-6]	0.19 (92)	0.16	0.03 (42)	0.05	0.005
c__TM7_[C-1]; o__TM7_[O-1]; f__TM7_[F-1]; g__TM7_[G-6]; s__sp._oral_taxon_870	0.19 (92)	0.16	0.03 (42)	0.05	0.005

These dramatically different species between the GC and HV groups are further analyzed by ROC curves, which indicate that *A. segnis*, *S. salivarius*, *M. micronuciformis* and *Oral taxon 870* achieve better diagnostic power with an AUC value greater than 0.75 ($p<0.001$) (Extended Data Fig. S2 A-D). Logistic regression is used to evaluate different combinations for distinguishing GC. The combination of *A. segnis* and *S. salivarius* yield an AUC value of 0.98 (95% CI 0.93 to 1.0, $p<0.001$) with 100% sensitivity and 91.7% specificity for distinguishing the GC patients from the HVs (Extended Data Fig. S2 E). The combination of *S. salivarius* and *Oral taxon 870* also achieved excellent performance with an AUC value of 0.93 (95% CI 0.82 to 1.0, $p<0.001$) for distinguishing the GC patients from the HVs (sensitivity: 91.7%, and specificity: 75%) (Extended Data Fig. S2 F). These results demonstrate that changes in the oral microbiota are associated with GC and have potential as cancer biomarkers.

Our previous studies demonstrated that GC could induce alterations in salivary protein glycopatterns, especially fucosylated glycoproteins, in the saliva of GC patients[13]. Here, a fucosylated neoglycoprotein (Fuc-BSA) and other neoglycoproteins (Gal-BSA and Man-BSA) are synthesized and their roles in the proliferation and adhesion of *A. segnis* are evaluated (Extended Data Fig. S3). The results showed that the proliferation of *A. segnis* is not affected by 3-100 μg/mL Fuc-BSA, Gal-BSA, Man-BSA, their monosaccharides (Fuc, Gal, and Man) or BSA (data not shown). In addition, 30 and 100 μg/mL Fuc-BSA significantly decreases the adhesion of *A. segnis* to CAL-27 cells (Fig. 3); however, 3 and 10 μg/mL Fuc-BSA and 30 and 100 μg/mL Man-BSA and Gal-BSA not affect this process (Extended Data Fig. S4A and S4B). A similar phenomenon is observed in HOEC cells, and 30 and 100 μg/mL Fuc-BSA also significantly decreases the adhesion of *A. segnis* to HOEC cells. These results demonstrate that the fucose moieties of proteins can disturb *A. segnis* attachment to the cell surface, which implies that they can prevent periodontal disease.

Among these seven bacteria, *A. segnis* and *M. micronuciformis* could be cultivated and showed increased abundance in the GC patients. *Haemophilus segnis* is first isolated in 1977[14] and reclassified into the *Aggregatibacter* genus in 2006[15]; it is a normal commensal in oral humans but is rarely reported as a pathogen. Infective endocarditis is the most well-known infection but is still rarely reported to be associated with *A. segnis*[16]. Periodontal disease, acute appendicitis, pancreatic abscess, and cholecystitis have also been associated with *A. segnis* on occasion[17,18]. Previous studies have focused mainly on case reports, and the true prevalence of this bacterium in human infections is probably underreported; the underlying mechanism associated with diseases is not yet explicitly known. To understand the related mechanisms, the glycopatterns of the outer membrane and total protein of *A. segnis* and *A. segnis* treated with 30 and 100 μg/mL Fuc-BSA are analyzed by lectin microarrays. There are 9 lectins (e.g., Jacalin, GSL-II, and SNA) that show decreased expression levels of the glycopatterns (e.g., Galβ1-3GalNAcα-Ser/Thr, GalNAcα-Ser/Thr and Sia2-6Gal/GalNAc), while there are 3 lectins (PHA-E, LEL, and MAL-I) that show extremely increased expression levels of the glycopatterns (Bisecting GlcNAc, high mannose-type N-glycans, and Galβ-1,4GlcNAc) on the outer membrane of *A. segnis* treated by Fuc-BSA (Extended Data Fig. S5A). However, 4 lectins (PHA-E, PNA, LEL and GSL-I) show increased

expression levels of glycopatterns (bisecting GlcNAc, Galβ1-3GalNAcα-Ser/Thr, high mannose-type N-glycans, and αGalNAc/αGal) in the total protein of *A. segnis* treated with Fuc-BSA (Extended Data Fig. S5B). These results implied that Fuc-BSA mainly affects glycopatterns on the outer membrane of *A. segnis*. Annotation of *A. segnis* in the carbohydrate-active enzymes database (CAZy) revealed that 24 putative glycosyltransferases (GTs) are distributed among 11 glycosyltransferase families. Of these, 8 GTs are associated with lipopolysaccharide (LPS) biosynthesis, 7 GTs are associated with cell wall formation, and others are associated with glycosylation, glycogen synthase, and carbohydrate metabolism (Extended Data Table S2)[19]. The qPCR results show that 3 mRNAs of LPS-associated GTs (NCTC10977_00605 (Rfaq_1), NCTC10977_00729, and NCTC10977_00649 (WaaA)) are significantly decreased and that the mRNA of mannosyltransferase OCH1 is significantly increased in *A. segnis* treated with Fuc-BSA (Fig. S7A and S7B), which indicate that Fuc-BSA interfered with the biosynthesis of core oligosaccharides and O-antigenic polysaccharides of LPS. These results suggest that Fuc-BSA mainly altered the glycopatterns of the outer membrane of *A. segnis*.

Second, *Candida albicans (C. albicans)* is the most prevalent human fungal pathogen causing infections and oral diseases in T2DM patients[20,21]. Our previous work showed that the expression level of the SAα2-3 structure in the saliva of T2DM patients is significantly lower than that of healthy people, which is related to T2DM susceptibility to avian influenza virus[10]. Here, sialoglycoproteins from bovine milk are used to inhibit the growth and adhesion of *C. albicans*[22]. The results show that their growth can be inhibited gradually by different concentrations (50, 100, 200, and 400 μg/mL) of sialoglycoproteins (Fig. 4A) and be inhibited entirely by 400 μg/mL sialoglycoproteins with an IC50 of 142.8 μg/mL (Fig. 4B). To determine the roles of Siaα2-3/6Gal glycans, 400 μg/mL desialoglycoproteins (sialoglycoproteins treated with sodium periodate), sialoglycoproteins without Siaα2-3Gal (sialoglycoproteins treated with α2-3 neuraminidase S), sialic acid, and BSA are also used to inhibit the growth of *C. albicans*. The results show that they cannot inhibit the growth of *C. albicans*, which implies that the Siaα2-3 moieties of sialoglycoproteins play a major role against *C. albicans*. Furthermore, 200 μg/mL sialoglycoproteins and desialoglycoproteins are used to blockade *C. albicans* to adhere to CAL-27 cells. Notably, sialoglycoproteins significantly inhibit the adhesion of *C. albicans* to CAL-27 cells; however, desialoglycoproteins cannot inhibit this process. These results also demonstrate that the Siaα2-3Gal moieties of sialoglycoproteins play a major role in inhibiting the adhesion of *C. albicans* to CAL-27 cells (Fig. 4D, E).

Currently, there are several processes that underlie the transition of a microbial community to a state of dysbiosis. In particular, diet can affect the community composition, and overexposure to dietary carbohydrates and host factors promotes the production of extracellular polymeric substances (EPS) and acidic metabolites, which cause the accumulation of acidogenic and aciduric microorganisms, thus driving the transition to a pathogenic biofilm community[23-25]. Bacteria can obtain nutrients from glycoproteins in saliva and gingival crevicular fluid during fasting[26,27]. Glycoproteins can be broken down into sugars and proteins and metabolized by bacteria that can gain energy to survive. During metabolism, sugars and proteins are further converted into acidic and basic small molecules, which can neutralize

each other to maintain a neutral state in the mouth. If sugar consumption is low and infrequent, the microbial communities on teeth remain stable. Despite being able to produce acids that demineralize enamel, the episodic pH decrease can be readily neutralized by saliva, which restores and maintains the mineralization of enamel[28]. However, once a community has transitioned to a dysbiotic state, the structural stability of functionally specialized components will allow the condition to persist for an extended period of time, promoting the development of oral diseases such as periodontitis and dental caries[29-31].

However, our studies demonstrated that human disease could induce different alterations in salivary protein glycopatterns[6]. The altered salivary protein glycopatterns (e.g., fucosylated or sialylated structures) induced by GC or T2DM are also drivers of dysbiosis of oral microbial communities and ultimately dental caries and periodontitis. The fucosylated neoglycoproteins and SAα2-3Gal structure can inhibit the growth and adhesion of *A. segnis* and *C. albicans* from the oral cavity of GC and T2DM, respectively. These findings provide a novel theory that dynamic communities of oral microbiota are regulated naturally by host salivary protein glycopatterns, having important implications for developing new carbohydrate drugs for oral and body health.

Methods

Study Population

The collection and use of human saliva samples for the research presented here were approved by the Ethical Committee of Northwest University (Xi'an, China), First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China). Written informed consent was received from participants for the collection of their saliva samples. This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. After a standardized endoscopic procedure and histopathological evaluation, individuals who were diagnosed with GC (N=12) were enrolled in this study. The age- and sex-matched HVs (N=12) were free of any precancerous lesions, as confirmed by gastroscopy. All the participants were ≥ 40 years old, and no significant differences were observed between the two groups regarding demographic, socioeconomic, and lifestyle characteristics. Patients who received preoperative radiotherapy, chemotherapy, chemoradiotherapy or antibiotic therapy were excluded from the study. The clinical characteristics of the HVs and GC patients are summarized in Extended Data Table S3.

Sequencing and Data Analysis

Bacterial genomic DNA was extracted by a Bacterial DNA Isolation Kit D3350-01 (OMEGA, USA) following the manufacturer's instructions. The 16S rDNA V3-V4 regions were amplified by specific bacterial primers 341F-806R with barcodes 341F: CCTAYGGGRBGCASCAG, and 806R: GGACTACNNGGGTATCTAAT. All PCRs were carried out with Phusion High-Fidelity PCR Master Mix (New England Biolabs, USA). The PCR products were extracted and quantified, pooled in equimolar concentrations and sequenced using the Ion S5^{TM} XL platform according to the manufacturer's recommendations. The methods for sequence

screening, diversity analysis and taxonomy-based analysis were performed as described in previous reports[32]. Differences between two arbitrary data sets were tested by the Wilcoxon test for each species using SPSS statistics 20.

Synthesis of neoglycoproteins

The synthesis of neoglycoprotein is divided into three steps (Extended Data Fig. S6A): the monosaccharides coupled with the linker (5-(4-hydroxyphenyl)pentanoic acid) to form glycan conjugates, and the carboxylates (-COOH) from glycan conjugates reacted to NHS in the presence of EDC, resulting in a semistable NHS, followed by reaction with amines (-NH2) of BSA to form amide crosslinks. Compared with the band of identically processed BSA, the upward migration bands of neoglycoproteins are obviously observed in the SDS-PAGE results (Extended Data Fig. S6B).

First, 5 μmol of each monosaccharide (fucose, mannose, and galactose (Sigma-Aldrich, USA)) was mixed with 2.5 μmol of 5-(4-hydroxyphenyl) pentanoic acid, dissolved in N,N-dimethylformamide (DMF), decreased to a pH under 3.0 using sulfuric acid, and then incubated at 60 °C for 12 h under gentle shaking with an appropriate amount of silicon dioxide. Second, after chloroform extraction, the glycan complexes were mixed with 10 μmol EDC and 25 μmol NHS in activation buffer (0.1 M MES (2-[morpholino] ethanesulfonic acid), 0.5 M NaCl, pH 6.0) and incubated at room temperature for 4 h. Third, the products were added to 100 nmol BSA solution (BSA dissolved in PBS), and then the buffer pH was adjusted to 7.4 and incubated at room temperature for 4 h. The Quench reaction was performed by adding hydroxylamine to a final concentration of 10 mM. The synthesized neoglycoproteins were dialyzed against PBS overnight and concentrated using an Amicon ultra-4 30 kDa ultrafiltration unit (Millipore, USA). The neoglycoprotein solutions were collected, filtered through 0.22 μm filters and stored at -80 °C. The synthesized neoglycoproteins were analyzed by 10% SDS-PAGE and then stained directly with alkaline silver.

Lectin Microarrays and Data Analysis

A lectin microarray was produced using 37 lectins with different binding preferences covering N- and O-linked glycans[9]. The Cy3-labeled glycoproteins or bacteria were incubated on a lectin microarray at 37 °C for 3 h with gentle rotation in the dark. The slides were washed three times with PBST and PBS, centrifuged dry and scanned immediately with 70% photomultiplier tubes and 100% laser power settings using a Genepix 4000B confocal scanner (Axon Instruments, USA). The acquired images were analyzed at 532 nm for Cy3 detection by Genepix 3.0 software. Differences between two arbitrary data sets or multiple data sets were tested by Wilcoxon test or one-way ANOVA for each lectin signal using SPSS statistics 20.

Real-Time PCR (qRT-PCR)

Total RNA was extracted using the Bacteria Total RNA Isolation Kit (Sangon Biotech, China) and converted into cDNA using the PrimeScript RT reagent Kit (TaKaRa, Japan). The cDNAs were then

subjected to real-time quantitative polymerase chain reaction (qRT-PCR) analysis (ABI ViiA^{TM} 7, Bio-Rad, USA) using gene-specific primers (Extended Data Table S4) and TB Green Fast qPCR Mix (TaKaRa, Japan). The 16S rRNA gene was used as an endogenous control for normalizing the relative expression of target genes.

Isolation of Sialoglycoproteins from Bovine Milk

According to the protocol in the literature[22]. In brief, serotonin-functionalized magnetic particle conjugates were thoroughly washed with binding buffer (20 mM Na_3PO_4, pH 6.0) and incubated sequentially with 1 mL of binding buffer containing 0.2 mL bovine milk with gentle shaking at room temperature for 3 h without light. The unbound proteins were removed from the conjugates with binding buffer until there were no detectable proteins. Finally, the sialoglycoproteins were eluted from the conjugates with eluting buffer (0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.3) and quantitated with the BCA method.

Destruction of Siaα2-3/6Gal Moieties and Enzymolysis of Siaα2-3 Gal Moieties of the Isolated Sialoglycoproteins

The Siaα2-3/6Gal structures of the isolated sialoglycoproteins were destroyed by treatment with 1 mM sodium periodate at 0 °C for 15 min, as described previously[33]. Then, sodium periodate was removed by using an ultracentrifugal filter (Ultracel-3k, Millipore Corp., Billerica, MA). The Siaα2-3 Gal moieties of the isolated sialoglycoproteins were digested by α2-3 neuraminidase S (New England Biolabs, USA) according to the manufacturer's recommendations.

Bacterial Strains and Cell Culture

A. segnis was obtained from the American Type Culture Collection (ATCC) and grown in 814 GC agar/broth medium supplemented with 15 μg/mL NAD^+ (Solarbio, Beijing, China) and 30 μg/mL vancomycin (Solarbio, Beijing, China) at 37 °C with 5% CO^2. *C. albicans ATCC 10231* was obtained from the National Center for Medical Culture Collections (CMCC) and aerobically grown at 37 °C in Sabouraud's dextrose broth supplemented with 50 mg/ml gentamicin (Solarbio, Beijing, China). CAL-27 and HOEC cells were obtained from Otwo Biotech Inc. (Shenzhen, China), cultured in DMEM (HyClone, Waltham, MA, USA) supplemented with 10% FBS (Gibco, Grand Island, NY, USA) and maintained at 37 °C with 5% CO^2.

Adhesion Assays

A. segnis or *C. albicans* were washed with fresh medium three times, centrifuged and diluted with fresh medium containing 100 μM FITC-d-Lys (Xiamen Bioluminor Bio-Technology, Xiamen, China). After 30 min of incubation at 37 °C, the cells were centrifuged, washed with fresh medium three times, and then resuspended in PBS. CAL-27 or HOEC cells (1×10^5 cells) were inoculated into confocal culture dishes (JingAn Biotechnology, Shanghai, China) and cultured in complete medium. Cells were grown to 60-70% confluence and then synchronized by serum starvation overnight. One hundred microliters of serum-free

DMEM with 5 μM DiD (AAT Bioquest, USA) was pipetted onto the dishes and gently agitated until all the cells were covered. After 30 min of incubation at 37 °C, the cells were washed three times with DMEM. *C. albicans* or FITC-labeled *A. segnis* (1×10^6 cells/mL) was added to the dishes in 500 μL serum-free DMEM, which was allowed to infect HOEC or CAL-27 cells for 45 min for adhesion. The infected cells were washed three times with 1× PBS and immobilized by incubating with 0.2% Triton X-100 in 4% paraformaldehyde for 30 min at RT. After DAPI staining (Thermo Fisher Scientific, Waltham, USA), a laser scanning confocal microscope FV 1000 (Olympus, Tokyo, JPN) was used to acquire the images with the merged channels of FITC (Ex/Em(nm)=488/520), DiD (Ex/Em(nm)=650/670) and DAPI (Ex/Em (nm)=358/461). A fluorescence microscope (Olympus, Tokyo, JPN) was used to observe the adhesion of *C. albicans* to cells.

References

1 Lamont, R. J., Koo, H. & Hajishengallis, G. The oral microbiota: dynamic communities and host interactions. *Nat Rev Microbiol* **16**, 745-759, doi:10.1038/s41579-018-0089-x (2018).

2 Fan, X. *et al.* Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study. *Gut* **67**, 120-127, doi:10.1136/gutjnl-2016-312580 (2018).

3 Flemer, B. *et al.* The oral microbiota in colorectal cancer is distinctive and predictive. *Gut* **67**, 1454-1463, doi:10.1136/gutjnl-2017-314814 (2018).

4 Long, J. *et al.* Association of oral microbiome with type 2 diabetes risk. *J Periodontal Res* **52**, 636-643, doi:10.1111/jre.12432 (2017).

5 Peters, B. A. *et al.* Oral Microbiome Composition Reflects Prospective Risk for Esophageal Cancers. *Cancer research* **77**, 6777-6787, doi:10.1158/0008-5472.Can-17-1296 (2017).

6 Zhang, J. *et al.* Identification of abnormal fucosylated-glycans recognized by LTL in saliva of HBV-induced chronic hepatitis, cirrhosis, and hepatocellular carcinoma. *Glycobiology* **29**, 242-259, doi:10.1093/glycob/cwy108 (2019).

7 Escapa, I. F. *et al.* New Insights into Human Nostril Microbiome from the Expanded Human Oral Microbiome Database (eHOMD): a Resource for the Microbiome of the Human Aerodigestive Tract. *mSystems* **3**, doi:10.1128/mSystems.00187-18 (2018).

8 Belda-Ferre, P. *et al.* The oral metagenome in health and disease. *The ISME journal* **6**, 46-56, doi:10.1038/ismej.2011.85 (2012).

9 Qin, Y. *et al.* Age- and sex-associated differences in the glycopatterns of human salivary glycoproteins and their roles against influenza A virus. *Journal of proteome research* **12**, 2742-2754, doi:10.1021/pr400096w (2013).

10 Zhong, Y. *et al.* Avian Influenza Virus Infection Risk in Humans with Chronic Diseases. *Scientific reports* **5**, 8971 (2015).

11 Shu, J., Yu, H., Li, X., Zhang, D. & Liu, X. Salivary glycopatterns as potential biomarkers for diagnosis of gastric cancer. *Oncotarget* **8**, 35718-35727 (2017).

12 Liu, X. *et al.* Salivary Glycopatterns as Potential Biomarkers for Screening of Early-Stage Breast Cancer. *EBioMedicine*, 70-79 (2018).

13 Shu, J. *et al.* Identification of N- and O-linked glycans recognized by AAL in saliva of patients with atrophic gastritis and gastric cancer. *Cancer Biomarkers* **22**, 669-681 (2018).

14 Kilian, M. A taxonomic study of the genus Haemophilus, with the proposal of a new species. *J Gen Microbiol* **93**, 9-62, doi:10.1099/00221287-93-1-9 (1976).

15 Nørskov-Lauritsen, N. & Kilian, M. Reclassification of Actinobacillus actinomycetemcomitans, Haemophilus aphrophilus, Haemophilus paraphrophilus and Haemophilus segnis as Aggregatibacter actinomycetemcomitans gen. nov., comb. nov., Aggregatibacter aphrophilus comb. nov. and Aggregatibacter segnis comb. nov., and emended description of Aggregatibacter aphrophilus to include V factor-dependent and V factor-independent isolates. *Int J Syst Evol Microbiol* **56**, 2135-2146, doi:10.1099/ijs.0.64207-0 (2006).

16 Revest, M., Egmann, G., Cattoir, V. & Tattevin, P. HACEK endocarditis: state-of-the-art. *Expert Rev Anti Infect Ther* **14**, 523-530, doi:10.1586/14787210.2016.1164032 (2016).

17 Welch, W. D., Southern, P. M., Jr. & Schneider, N. R. Five cases of Haemophilus segnis appendicitis. *J Clin Microbiol* **24**, 851-852, doi:10.1128/jcm.24.5.851-852.1986 (1986).

18 Carson, H. J., Rezmer, S. & Belli, J. Haemophilus segnis cholecystitis: a case report and literature review. *The Journal of infection* **35**, 85-86, doi:10.1016/s0163-4453(97)91193-2 (1997).

19 Whitfield, C. & Trent, M. S. Biosynthesis and export of bacterial lipopolysaccharides. *Annu Rev Biochem* **83**, 99-128, doi:10.1146/annurev-biochem-060713-035600 (2014).

20 Harriott, M. M. & Noverr, M. C. Importance of Candida-bacterial polymicrobial biofilms in disease. *Trends in microbiology* **19**, 557-563, doi:10.1016/j.tim.2011.07.004 (2011).

21 Casarin, R. C. *et al.* Subgingival biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis. *J Periodontal Res* **48**, 30-36, doi:10.1111/j.1600-0765.2012.01498.x (2013).

22 Wang, X. *et al.* Purification of sialoglycoproteins from bovine milk using serotonin-functionalized magnetic particles and their application against influenza A virus. *Food & Function* **11**, 6911-6920, doi:10.1039/D0FO01447H (2020).

23 Bowen, W. H., Burne, R. A., Wu, H. & Koo, H. Oral Biofilms: Pathogens, Matrix, and Polymicrobial Interactions in Microenvironments. *Trends in microbiology* **26**, 229-242, doi:10.1016/j.tim.2017.09.008 (2018).

24 Marsh, P. D. & Zaura, E. Dental biofilm: ecological interactions in health and disease. *Journal of clinical periodontology* **44 Suppl 18**, S12-s22, doi:10.1111/jcpe.12679 (2017).

25 Takahashi, N. & Nyvad, B. The role of bacteria in the caries process: ecological perspectives. *Journal of dental research* **90**, 294-303, doi:10.1177/0022034510379602 (2011).

26 Dabdoub, S. M., Ganesan, S. M. & Kumar, P. S. Comparative metagenomics reveals taxonomically idiosyncratic yet functionally congruent communities in periodontitis. *Scientific reports* **6**, 38993, doi:10.1038/srep38993 (2016).

27 Lu, M., Xuan, S. & Wang, Z. Oral microbiota: A new view of body health. *Food Science and Human Wellness* **8**, 8-15, doi:https://doi.org/10.1016/j.fshw.2018.12.001 (2019).

28 Tanner, A. C. R., Kressirer, C. A., Rothmiller, S., Johansson, I. & Chalmers, N. I. The Caries Microbiome: Implications for Reversing Dysbiosis. *Advances in dental research* **29**, 78-85, doi:10.1177/0022034517736496 (2018).

29 Mark Welch, J. L., Rossetti, B. J., Rieken, C. W., Dewhirst, F. E. & Borisy, G. G. Biogeography of a human oral microbiome at the micron scale. *Proc Natl Acad Sci U S A* **113**, E791-800, doi:10.1073/pnas.1522149113 (2016).

30 Suwannakul, S., Stafford, G. P., Whawell, S. A. & Douglas, C. W. I. Identification of bistable populations of Porphyromonas gingivalis that differ in epithelial cell invasion. *Microbiology (Reading, England)* **156**, 3052-3064, doi:10.1099/mic.0.038075-0 (2010).

31 Valm, A. M. *et al.* Systems-level analysis of microbial community organization through combinatorial labeling and spectral imaging. *Proc Natl Acad Sci U S A* **108**, 4152-4157, doi:10.1073/pnas.1101134108 (2011).

32 Yang, K. *et al.* Microbial diversity in combined UAF–UBAF system with novel sludge and coal cinder ceramic fillers for tetracycline wastewater treatment. *Chemical Engineering Journal* **285**, 319-330, doi:https://doi.org/10.1016/j.cej.2015.10.019 (2016).

33 Kim, D. S. *et al.* Both α2,3- and α2,6-linked sialic acids on O-linked glycoproteins act as functional receptors for porcine Sapovirus. *PLoS Pathog* **10**, e1004172, doi:10.1371/journal.ppat.1004172 (2014).

Figures

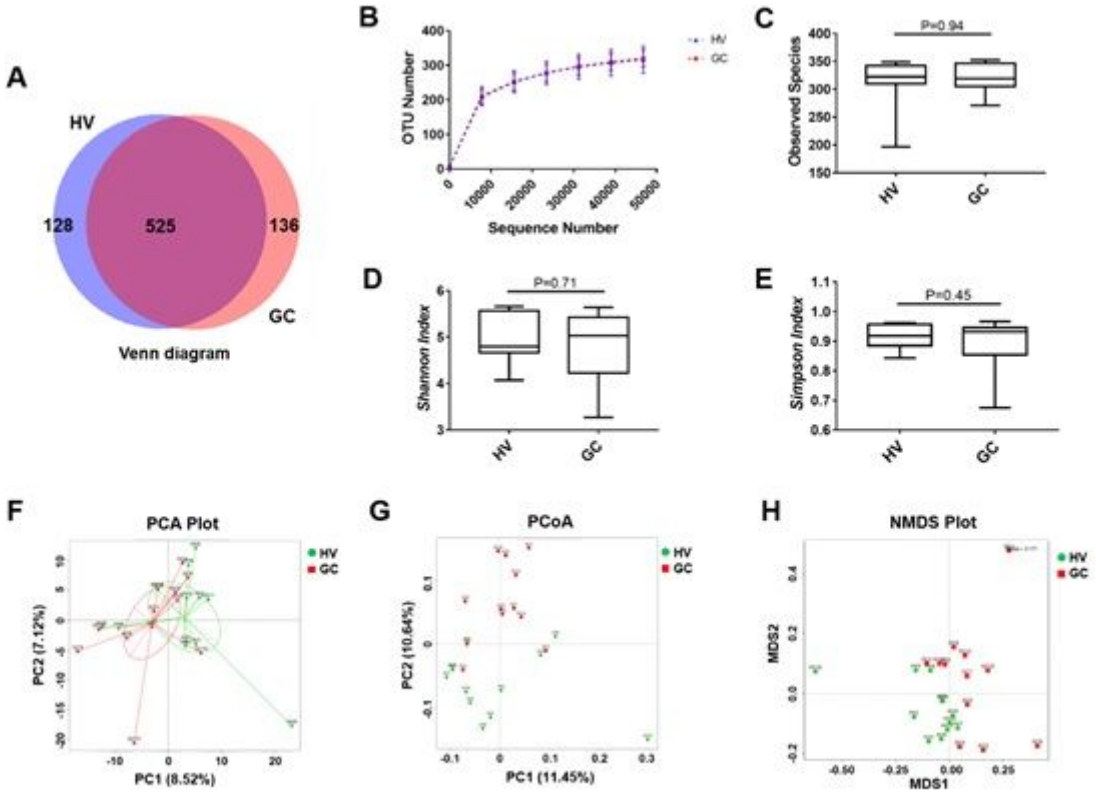

Figure 1

Comparison of the structures of the oral microbiota between the HV and GC groups. (A) Venn diagram illustrating the overlap of the species identified in the oral microbiota of the HV and GC groups, (B) OTU numbers, (C) Observed species, (D) Shannon index, (E) Simpson index, (F) PCA, (G) PCoA, (H) NMDS analysis.

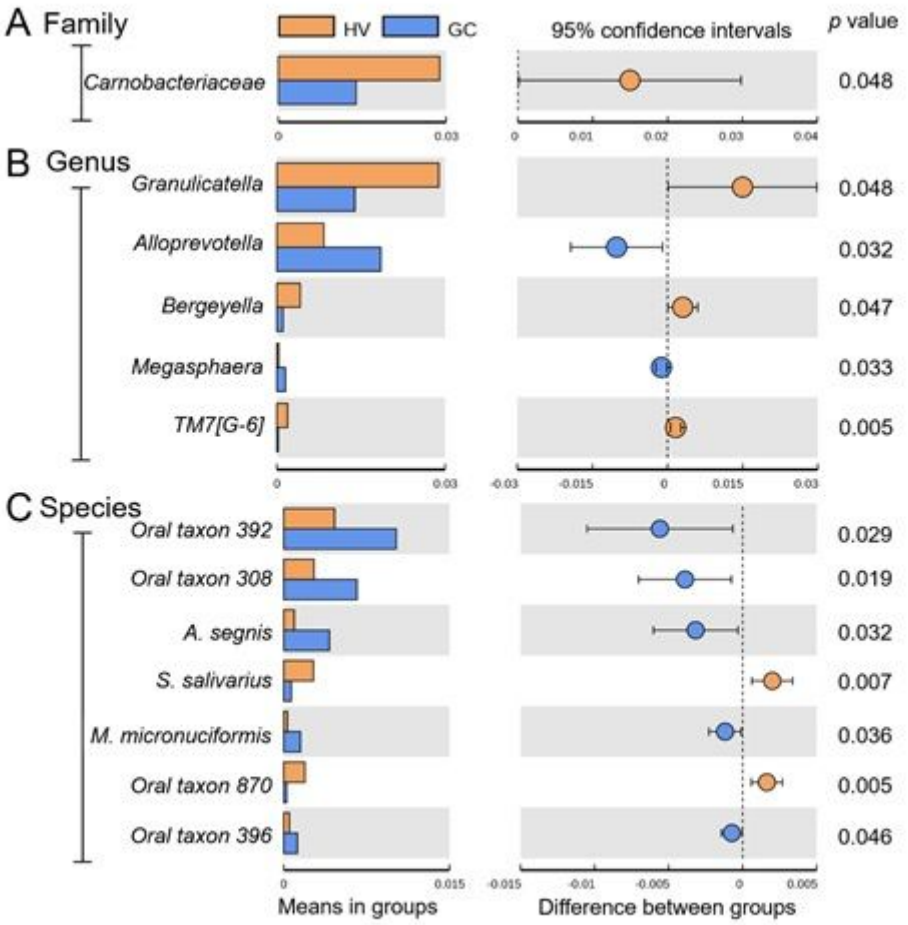

Figure 2

Comparison of the relative abundance at the bacterial family (A), genus (B) and species (C) levels in the HV and GC groups.

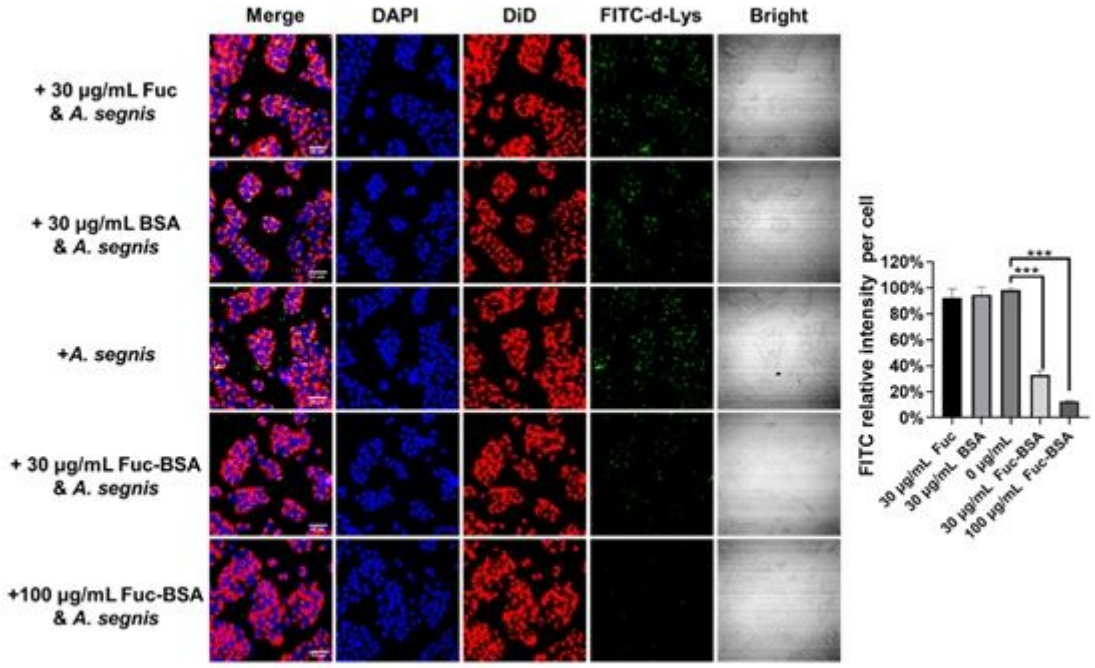

Figure 3

The adhesion of A. segnis to HOEC cells. A. segnis is added to HOEC cells with Fuc-BSA, BSA or Fuc and allowed to infect for 90 min. Fuc-BSA (30 and 100 μg/mL) significantly decreased the adhesion of A. segnis to HOEC cells. The images are acquired under the same conditions for the merged channels of FITC (Ex/Em(nm)=488/520), DiD (Ex/Em(nm)=650/670) and DAPI (Ex/Em (nm)=358/461). Statistical significance analysis for FITC fluorescence intensity per cell is computed by one-way ANOVA (*: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$).

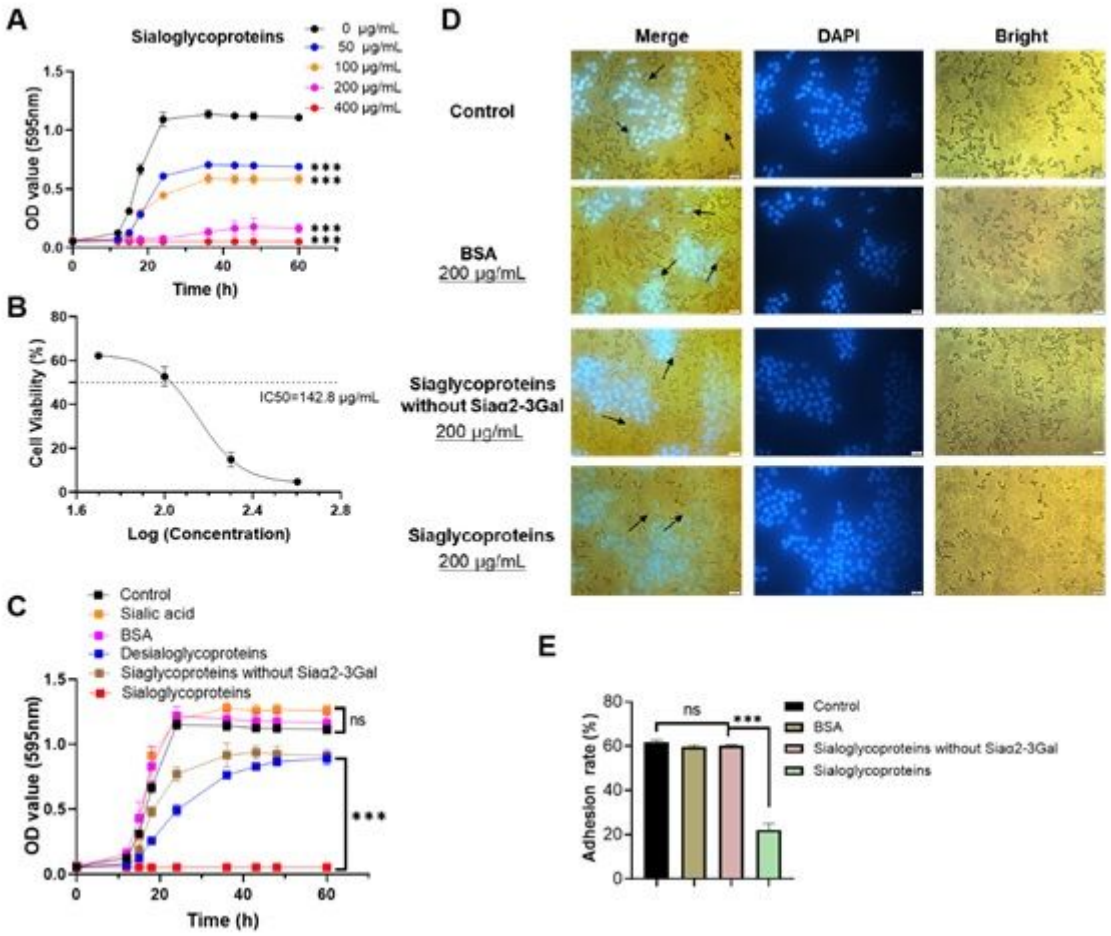

Figure 4

The growth and adhesion of C. albicans are affected by Sia2-3Gal moieties. (A) Effects of different concentrations of sialoglycoproteins on the growth of C. albicans. (B) The IC50 of sialoglycoproteins against C. albicans. (C) The effects of sialic acid, desialoglycoproteins and sialoglycoproteins without Siaα2-3Gal on the growth of C. albicans. Destruction of Siaα2-3 moieties significantly affect the inhibition activity of sialoglycoproteins against C. albicans, indicating that the Siaα2-3 moieties play a major role in the inhibition progress. (D) The effects of sialoglycoproteins and sialoglycoproteins without Siaα2-3Gal limitation on the adhesion of C. albicans to CAL-27 cells. (E) The adhesion rate of C. albicans to CAL-27 cells.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ExtendedDataTableS1.rtf
- ExtendedDataTableS2.rtf
- ExtendedDataTableS3.rtf

- ExtendedDataTableS4.rtf
- ExtendedDataFigures.pdf