Analysis of Oral Microbiome on Temporary Anchorage Devices Under Different Oral Hygiene Conditions

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Research Article

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Abstract

Background: Temporary anchorage devices (TADs) are maximum anchorages that have been widely used in orthodontic treatment. Poor oral hygiene might result in inflammation and decreased stability of the TADs. In this study, we aimed to unveil the differences in the microbiome between TADs under different oral hygiene conditions.

Methods: Oral hygiene condition was stratified by Oral Hygiene Index- Simplified (OHI-S), Plaque Index (PLI), and Gingival Inflammation. Scanning electron microscopy (SEM) was used to analyze the existence of biofilm on the surface of 8 TADs. Ten TADs from the good oral hygiene group (GOH), and 10 TADs from the poor oral hygiene group (POH) were analyzed by 16S rRNA gene sequencing.

Results: Principal coordinate analysis (PCoA) based on β diversity revealed differential sample clusters depending on oral hygiene conditions. When comparing specific genera, Veillonella, Streptococcus, Neisseria, were more enriched in the poor oral hygiene group. Conversely, Fusobacterium, Porphyromonas exhibited more richness in the good oral hygiene group. TADs in the good oral hygiene group demonstrated enriched microbial activities involved with signal transduction, cell mobility and energy metabolism. TADs in poor oral hygiene demonstrated enriched functions in membrane transport, transcription and signaling molecules and interactions.

Conclusions: In summary, this analysis elucidated the difference in total composition and function of TADs oral microorganisms between patients with good oral hygiene and patients with poor oral hygiene, which highlighted the importance of maintaining good oral hygiene in TADs treatment.

Background

Anchorage control has been a major concern for orthodontists for decades. The effectiveness of traditional extraoral anchorage relies greatly on patients’ obedience, while intermaxillary and intramaxillary anchorage might cause unwanted movement of the anchorage tooth. As “maximum anchorage”, the temporary anchorage devices (TADs) have been well received since their appearance. Not only does this device facilitate anchorage reinforcement compared to conventional anchorage control, but it also has great advantages in flexibility, versatility, control, and minimal invasiveness\(^1\),\(^2\). It is particularly indicated in anterior en-masse retraction, molar protraction, as well as the intrusion of supr-erupted teeth, and midline correction\(^3\).

The success rate of TADs is generally over 80\(^\%\)\(^4\). However, on some occasions, TADs present excessive mobility which eventually leads to loss of anchorage. Factors affecting the success rate of TADs may be divided into location-related factors (bone quality, mucosa, position), orthodontic-related factors (force application), and implant-maintenance factors (the presence of inflammation)\(^5\). Oral hygiene condition is one of the decisive factors of inflammation and reflects implant-maintenance condition\(^5\)–\(^7\). Poor oral hygiene induces changes in the surrounding environment, which eventually influences the colonization of TADs surrounding bacteria. Studies targeting peri-implantitis have unveiled the pathogenic role of
bacteria when attached to the surface of a dental implant if oral hygiene methods were not properly introduced[8]. TADs and dental implants share a common denominator in being titanium implants in the oral cavity. When a TAD is inserted trans-gingivally and exposed to all sorts of oral bacteria, an artificial sulcus is created, which favors microorganisms migration and biofilm formation[9]. Until now, a few epidemiological surveys have listed oral hygiene as the most significant factor affecting TADs success[10, 11]. Even though other clinical trials have not confirmed the direct effect of poor oral hygiene on the success rate, they have highlighted the strong role of local inflammation in increasing the risk of failure[5–7, 12].

Current studies concerning the microbiome effects on TADs have concentrated on the observation of attached microflora and the identification of certain species. Ferreira et al utilized a scanning electron microscope (SEM) to observe the microbiota attached to the surface of the TADs, revealing biofilm existence on the head, transmucosal, and body segments of the TADs. With the utilization of fluorescence imaging, Garcez et al observed higher fluorescent intensity in inflamed TADs with redness on surrounding tissues[13]. When exploring the differences of microbiome between stable TADs and unstable TADs, nevertheless, polymerise chain reaction (PCR) and DNA-DNA hybridization revealed no major differences in the tested microbiome. These studies partly uncovered the role of microbiomes in generating TADs surrounding diseases but failed to build up the complete connection. Moreover, these studies did not demonstrate the overall structure of biofilm and neglected the immune response from the human host. The emergence of next-generation sequencing (NGS) technology has created a new way for microbiome research, which can be used to detect population diversity, identify the structure of microbiome and predict functional roles on certain occasions[14]. Therefore, 16S rRNA gene sequencing would be an effective tool in assessing the microbiome on the TADs surface.

Therefore, here, we brought up the hypothesis that oral hygiene condition influences microbiota composition on the TADs surface. We conducted a case-control study to investigate the structural and compositional changes in oral microbiota on TADs depending on different oral hygiene conditions.

**Methods**

**Participant Selection and Sample Collection**

All of the subjects in this study participated in orthodontics treatments in Peking University Hospital of Stomatology. In all of them, the use of orthodontic anchorage was indicated. Each participant signed an informed consent form to enroll in the trial. This analysis was ratified by the Ethics Committee of the Peking University Hospital of Stomatology under PKUSSIRB-202060204. All methods were carried out in accordance with relevant guidelines and regulations.

All subjects selected met the following criteria: 1) aged 12–45 years; 2) periodontally healthy or received systematic periodontal treatment before orthodontic treatment. Before placement of the appliance,
patients with a periodontal probing depth of less than 3 mm were included; 3) non-smokers; 4) without systematic disease; 5) not pregnant; 6) no antibiotics used up to three months before removal.

Self-drilling titanium orthodontic TADs (diameter, 1.5 mm; length, 7 mm or 8 mm; Zhongbang Medical Treatment Appliance, Xi'an, China) were inserted in the maxilla, between tooth roots of anterior or posterior teeth, between the buccal or palatal surface. All TADs were inserted by one experienced orthodontist. No damage to the adjacent tooth roots was observed. All patients received oral hygiene instructions to brush TADs and the surrounding tissues when adopting oral hygiene methods. All TADs were activated 1 month after placement. In total, 8 TADs from 8 individuals, 4 from the good oral hygiene group (GOH), and 4 from the poor oral hygiene group (POH) were included for SEM. Twenty TADs from 20 patients, 10 from GOH, and 10 from POH were included for 16S rRNA gene sequencing. All of the included TADs remained stable during treatment and were removed until attaining the desired result.

**Oral Hygiene stratification**

Clinical parameters of the oral hygiene condition were obtained when the TADs were removed. Oral hygiene conditions were measured by Oral hygiene Index- Simplified (OHI-S)[15], Plaque Index (PLI) [16] as well as gingival inflammation. The Index teeth of the OHI-S and PLI were 16,11,26,31,36, and 46. The clinical parameter score of each patient was determined by the average measured value of the designated tooth. Gingival inflammation was evaluated by calculating the number of swollen dental papillae in the whole oral cavity, as follows: 0, normal gingiva; 1, slight edema in no more than two dental papillae; 2, redness, edema, and glazing in no more than two dental papillae; 3, redness, edema, and glazing in more than two dental papillae and no more than five dental papillae; 4, redness, edema, and glazing in more than five dental papillae and no more than eight dental papillae; and 5, redness, edema, and glazing in more than eight dental papillae.

GOH group was defined as a tiny amount of dental plaque on the index teeth and only minor signs of gingival inflammation. POH group was defined as a massive amount of dental plaque on the index teeth and marked signs of gingival inflammation.

To be more specific, the GOH group referred to OHI-S no more than 1, PLI less than 1, and Gingival Inflammation no more than 2. Conversely OHI-S more than 1, PLI no less than 2, and Gingival Inflammation no less than 3 was defined as POH group.

**SEM Analysis**

Eight TADs were observed under SEM. After removal, the TADs were transferred to 1.5 mL nonpyrogenic microcentrifuge tubes containing 0.5 mL 4% glutaraldehyde and stored at −4°C. Before observation, the TADs were dehydrated in increasing concentrations of ethanol (30%, 50%, 70%, 80%, 90%, 95%, and 100%). After conductive coating, they were sent for examination under SEM.

**DNA Extraction**
The TADs were placed in nonpyrogenic microcentrifuge tubes containing 0.5 mL normal saline solution and stored at −20°C refrigerator temporarily. Before DNA extraction, the tubes were agitated in an ultrasound cleaner (SB-3200DTN, Scientz, Ningbo, China) for 20 minutes. The tubes were then centrifuged at 8000 rpm for 15 minutes to remove the supernatant. The precipitate was then sent for DNA extraction.

QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) was used to extract genome DNA. Extraction procedures were performed according to the kit instructions. Before extraction, 180 µL lysozyme (Solarbio, Beijing, China) was added to the reaction system. The system was then incubated at 37°C for 30 minutes. NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Carlsbad, CA, United States) and 1% agarose gel electrophoresis were used to determine the purity and integrity of DNA.

### 16S rRNA Gene Sequencing

Primer 341F (5'-CCTACGGGRSGCAGCAG-3') and 806R (5'- GGACTACVVGGGTATCTAATC-3') were added to amplify the 16S rRNA hypervariable V3-V4 region. Unique index and adapter sequence were added to the 5' to distinguish each sample. Next, the KAPA HiFi Hotstart ReadyMix PCR kit (Kapa Biosystems, Wilmington, MA, USA) was utilized to perform PCR. Then, AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was used to purify the amplicon. The library was quantified by Qubit (Thermo Fisher Scientific, Carlsbad, CA, United States) and real-time PCR with the same proportion. Miseq PE250 platform (Illumina, San Diego, CA, USA) was adopted for sequencing.

### Data Processing

Raw data were deposited at Sequence Read Archive under project no. PRJNA690665. Preprocessing of data was performed under guidance [17]. After demultiplexing, Vsearch [18] (version 2.15) was used to merge raw paired-end sequences according to the overlap of the paired-end reads, allowing for a maximum of five mismatches. Barcode and primers were then removed allowing a maximum error rate of 1% by Vsearch [18] (version 2.15) to obtain clean reads. After dereplication, unoise3 in USEARCH [19] was used to denoise to amplicon sequence variance (ASV) for representative sequence selection. Next, Vsearch [18] (version 2.15) was utilized to detect and exclude chimeras. The feature table was created by using Vsearch [18] (version 2.15). RDP classifier and the Human Oral Microbiome Database[20, 21] were both employed as databases in sequence annotation.

To start the downstream analysis, random rarefaction procedures were taken for each pre-processing sequence to mitigate the effect of varying sequencing depths. α-diversity indices (the Chao1 richness estimator, Shannon index) were calculated as metrics to the microbial diversity within each sample. Bray Curtis distance and Unifrac distance were assessed as representations of the overall microbiome dissimilarities or β-diversity. Principal coordinate analysis (PCoA) was implemented to reflect the β-diversity through R. Then, ASVs were classified into microbial taxa (phylum, class, order, family, and genus). The phylogenetic tree was constructed on ITOL (https://itol.embl.de/). Linear discriminant analysis Effect Size (LEfSe) was used to identify differentially taxa between groups[22]. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)[23] (version 1.1.3) tool...
was adopted to predict functional roles based on the Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway.

**Statistical Analysis**

An independent sample Student's $t$-test and nonparametric Wilcoxon's test were used to evaluating demographic features and clinical parameters between the two groups. The difference between $\alpha$-diversity was calculated by analysis of variance (ANOVA). The pairwise permutational multivariate analysis of variance (PERMANOVA) procedure tested the significance of $\beta$-diversity between TADs with good or poor oral hygiene. This was realized by the Adonis function of the R package vegan 2.5-6, allowing for 9999 permutations. Edge R test was used to determine differences between ASVs. Bar plots and cladogram graphs served as visualizations of LEfSe results (http://huttenhower.sph.harvard.edu/galaxy/). Spearman's correlation coefficients were calculated to measure the correlation between bacteria. Cytoscape (ver. 3.5.1) was used to visualize the correlation network between genera. LEfse test was also employed to identify statistically different KEGG pathways between the two groups.

**Data Availability Statements**

Raw data were deposited at Sequence Read Archive under project no. PRJNA690665.

**Results**

**Overview of Subjects and Samples**

In this study, we performed SEM observation on 8 samples from 8 individuals. Then, we conducted 16S rRNA gene sequencing of 20 TADs samples from 20 individuals.

The individuals’ demographic features and clinical parameters were presented in Table 1 for 16S rRNA sequencing analysis. Similarly, those features for samples involving SEM were also listed in Additional File 1. Significance was observed in OHI-S, PLI, and Gingival Inflammation in the two tables.
Table 1
Demographic and clinical features of subjects under sequencing

| Variable                  | GOH (n = 10) | POH (n = 10) | significance |
|---------------------------|--------------|--------------|--------------|
| Age (y)                   | 27 ± 5.18    | 21.8 ± 9.85  | ns           |
| Sex Ratio (Male: Female)  | 1:9          | 2:8          | ns           |
| Time in Oral Cavity (d)   | 681.6 ± 294.85 | 683.6 ± 329.10 | ns           |
| OHI-S                     | 0.56 ± 0.25  | 1.46 ± 0.37  | *            |
| Plaque Index              | 0.84 ± 0.18  | 2.72 ± 0.64  | *            |
| Gingival Swelling         | 1.3 ± 0.48   | 4.6 ± 0.70   | *            |

*Significant difference between GOH and POH
ns: No significant difference between GOH and POH

SEM Proving the Existence of Bacteria

To explore the microbiome on the TADs surface under different oral hygiene conditions, we first performed SEM to prove the existence of the microbiome on TADs (Fig. 1). SEM demonstrated the existence of microflora on the surface of observed TADs, both in GOH and POH. Rods and coccoid bacteria were all seen in this region. Besides, tissue remnants containing fibers and red blood cells were also observed. This testified microbiome colonization on the surface of TADs.

Phylogenetic Alterations under Different Oral Hygiene Conditions

During 16S rRNA gene sequencing processing, a total of 491600 clean reads were acquired. The average sequences for each sample were 24580, eliciting 960 ASVs.

To characterize the microbiome of the individuals with poor oral hygiene, α-diversity and β-diversity were first evaluated as reflections of the overall structural features and composition (Fig. 2). Although TADs in individuals with poor oral hygiene presented higher α-diversity reflected by Chao1 and Shannon, no statistical difference was observed (p = 0.874, p = 0.685, respectively) (Fig. 2a, Fig. 2b). However, PCoA based on weighted UniFrac distances revealed a statistically significant discrepancy in phylogenetic structures between the GOH and POH (p = 0.004) (Fig. 2c). Different clusters were formed, indicating separation in microbiome composition between the groups. Similarly, PCoA based on Bray Curtis distances revealed a clear separation between the groups (p = 0.017) (Fig. 2d).

Identification of Microbiota Composition

Next, we classified ASVs to certain microbial taxa to identify the compositional changes in individuals under different oral hygiene conditions. In general, we discovered 12 phyla, 23 classes, 33 orders, 54
families, and 97 genera. Phylum, such as *Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria*, constituted the majority of the microbiota on the TADs (Fig. 2e, Fig. 3). Genera including *Fusobacterium, Veillonella, Prevotella, Streptococcus, Porphyromonas, Neisseria*, and *Saccharibacteria_TM7 (G-1)* predominated (Fig. 2f, Fig. 3). ASVs from TADs from individuals with poor oral hygiene were compared with TADs with good oral hygiene. In total, 149 ASVs were depleted, and 165 ASVs were enriched (Additional File 2a). Differential ASVs belonged to different phyla characterized in Supplementary Fig. 1B (Additional File 2b). Differential ASVs were mostly seen in *Actinobacteria, Bacteroidetes, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Saccharibacteria_(TM7)* (Additional File 2c).

To better characterize the differences, the LEfSe test was used. The cladograms revealed the hierarchical relationship of those bacteria (Fig. 4a). The bar plot showed the significance of the differential microbiome (Fig. 4b). At the genus level, *Veillonella, Streptococcus, and Neisseria* were more enriched in the poor oral hygiene group. Conversely, *Fusobacterium, Porphyromonas* exhibited more richness in the good oral hygiene group (Fig. 4c). When comparing specific species, however, *Veillonella dispar* and *Streptococcus mutans* showed higher abundance in the POH group, while *Fusobacterium nucleatum* and *Porphyromonas gingivalis* did not differ significantly between groups (Additional File 3).

**Correlation Networks of Microbiome on the TADs**

Bacteria showed strong interactions with each other. In order to explore the connection between the most abundant genera, we then generated abundance-based correlation networks (Fig. 5). From the correlation network, GOH enriched genera (*Fusobacterium, Porphyromonas, Saccharibacteria_TM7 (G-1), Alloprevotella, Bacteroidaceae_[G-1], Fretibacterium, Catonella, and Lachnospiraceae_[G-7]*) and POH enriched genera (*Veillonella, Streptococcus, Neisseria, Cardiobacteria, Rothia, and Kingella*) clustered into separate modules, showing positive correlations within each group. Notably, POH enriched genera demonstrated negative correlations with GOH enriched genera, indicating differences and separations in connections.

**Microbiota Involvement in Functional Variation**

To study the functional changes in TADs in individuals with poor oral hygiene, the PICRUSt algorithm was employed to predict the path of microbiota derivation based on the KEGG database. Differences in functional abundance between TADs with good oral hygiene and TADs with poor oral hygiene were evaluated (Fig. 6). TADs in the good oral hygiene group demonstrated enriched microbial activities involved with signal transduction, cell mobility and energy metabolism. TADs in poor oral hygiene demonstrated enriched functions in membrane transport, transcription and signaling molecules and interactions.

**Discussion**
Our study characterized alterations in microbial community profiles on the TADs surface depending on the oral hygiene condition. We identified the compositional and phylogenetic changes in the microbiome on the surface of TADs in relation to the oral hygiene condition. We also predicted the correlation between microbiome colonization and functional involvement. To the best of our knowledge, this is pioneering research in understanding how oral hygiene influences microbiome colonization on the surface of TADs. This is also the first article that characterizes the microbiome on TADs with next-generation sequencing.

Microbial will colonize on the surface of TADs. When a TAD is inserted, a new site is created, which is defined as the gingival sulcus between the surrounding gingiva and the TAD cervical [24]. In a previous study utilizing SEM, Ferreira discovered bacteria colonization on the head, transmucosal surface, and body segment of TADs[25]. Similarly, in our study, the existence of microflora was observed on the surface of TADs. Previous studies also observed the adhesion, aggregation, and development of the microbial colonization process in TADs using cell growth methods or fluorescence images[13, 24]. The interactions between the microorganisms and the host maintain the microecological balance around the TADs[9].

There is strong evidence that patients who failed to control plaque at a low level after implant surgery have an increased risk of peri-implantitis. Resembling this process, if oral hygiene methods could not be implemented properly, inflammation around TADs is generated. As local inflammation contributes to the increased risk of TADs failure, the longevity of the TADs is eventually compromised[5–7, 12]. In our study, TADs in the good oral hygiene and poor oral hygiene groups displayed compositional and phylogenetic differences. Genera *Veillonella, Streptococcus, Neisseria* were significantly elevated in the POH group. *Streptococcus* species, especially acidogenic and aciduric species, such as *Streptococcus mutans*, are linked to the progression of dental caries [26, 27]. It can adhere to the solid surface and promote interactions with other microorganisms. *Veillonella* has been frequently detected in the microflora of individuals with poor oral hygiene conditions, and it has also been elevated in the microbiota of patients with dental caries and periodontitis[28]. In the pathogenesis of dental caries, the presence of *Veillonella* can promote the formation of biofilms of *Streptococcus mutans, Streptococcus gordon,* and *Streptococcus salivarius*[29]. In periodontitis, the adhesion of *Veillonella* and *Porphyromonas gingivalis* in the process of biofilm formation can produce a synergistic effect[30]. In summary, a higher proportion of *Streptococcus, Veillonella,* and *Neisseria* may be an indicator of poor oral hygiene conditions. Although, in the good oral hygiene group, genera *Fusobacterium, Porphyromonas,* were enriched. *Fusobacterium nucleatum* and *Porphyromonas gingivalis* did not differ significantly between groups. These species were gram-negative species that populate the subgingival crevice of the mouth. We speculated that in good oral hygiene, supragingival bacteria that habit on the exposed solid surfaces were wiped off promptly. This led to a higher proportion of subgingival bacteria. Moreover, previous studies targeting stable and unstable TADs unveiled a slight elevation, though not significant, in the prevalence and quantification of these periodontopathic pathogens in successful TADs [31], which indicate the presence of periodontal pathogens alone might not induce significant changes to the local environment towards TADs.
Characterizing microbiome function is necessary to broaden our knowledge of how oral hygiene affects the microbiome on the TADs’ surface. We used PICRUSt as a substitution method to characterize functional changes, which has been implemented in other sequencing studies[32–34]. Microbial activities involved with membrane transport, transcription and signaling molecules and interactions were abundant in the POH group. In dental caries, vigorous microbial metabolism was indicated in the oral bacterial community[34]. In periodontal disease, genes of pathogenic activities were detected in functional prediction, including bacterial motility, energy metabolism, and lipopolysaccharide biosynthesis[32, 33]. Nevertheless, in our study, the POH group demonstrated vigorous metabolic activities. No difference among these clearly defined pathogenic functions was found, indicating a rather mild change in microbial function in poor oral hygiene situations. Demonstrating the role of the key bacteria that encode these functions and setting up the link between these functions and the mobility of TADs will be crucial in future research.

Our study discussed the relationship between oral hygiene and microbiomes on the TADs surface using next-generation sequencing. Meanwhile, our study had a few limitations. The surgical technique applied in the extraction of TADs would unavoidably contact the adjacent soft tissue. This contact might lead to the induction of a small amount of bacterial DNA from other sites of the oral cavity. Besides, the TADs samples were difficult to acquire, and the sample size was relatively limited. Last, each individual also exhibited individual variance in the oral microbiome composition. Considering that oral hygiene affects TADs surrounding microenvironment and could potentially influence the success of TADs, further researches are needed to explain the mechanism of the oral microbiome and its relations with immobility.

**Conclusions**

This analysis elucidated the difference in total composition and function of TADs oral microorganisms between patients with good oral hygiene and patients with poor oral hygiene, which highlighted the importance of maintaining good oral hygiene in TADs treatment.

**Abbreviations**

TADs: Temporary anchorage devices; OHI-S: Oral Hygiene Index- Simplified; PLI: Plaque Index; SEM: Scanning electron microscopy; PCoA: Principal coordinate analysis; PCR: Polymerise chain reaction; NGS: Next-generation sequencing; ASV: Amplicon sequence variance; LEfSe: Linear discriminant analysis Effect Size; PICRUSt: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

**Declarations**

**Acknowledgements**

Not applicable

**Author’s Contribution**
NZ and QZ participated in collecting the samples, performing the experiments, analyzing the data, and drafting the manuscript. XW contributed to designing the study, collecting the samples and revising the manuscript. YG and SC collected samples, YT and YZ participated in revising the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available in the Sequence Read Archive repository (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA690665) under project no. PRJNA690665.

Ethics approval and consent to participate

Ethical approval for this study was obtained by the Ethics Committee of the Peking University Hospital of Stomatology under PKUSSIRB-202060204. Each participant signed an informed consent form to enroll in the trial. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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**Figures**

a. The whole picture of the TAD. b. Higher magnification of new sterilized TAD×5.00k. c. Higher magnification of the transmucosal segment of GOH TAD with tissues and biofilm formation ×5.00k. d. Higher magnification of body segment of GOH TAD with bacteria ×5.00k. e. Higher magnification of the tip of BOH TAD with tissues and biofilm formation ×5.00k. f. Higher magnification of the tip of BOH TAD with bacteria ×5.00k.

**Figure 1**

SEM images of TADs showing microbiome colonization on its surface. a. The whole picture of the TAD. b. Higher magnification of new sterilized TAD×5.00k. c. Higher magnification of the transmucosal segment of GOH TAD with tissues and biofilm formation ×5.00k. d. Higher magnification of body segment of GOH TAD with bacteria ×5.00k. e. Higher magnification of the tip of BOH TAD with tissues and biofilm formation ×5.00k. f. Higher magnification of the tip of BOH TAD with bacteria ×5.00k.
Figure 2

Comparisons of α and β diversity between GOH and POH microbial communities. a. A boxplot of α-diversity Chao 1 index between groups (p=0.874). b. A boxplot of α-diversity Shannon index between groups (p=0.685). c. Principal coordinate analysis (PCoA) based on weighted UniFrac distances is shown for the GOH (green) and POH (pink) (p=0.004). d. Principal coordinate analysis (PCoA) based on Bray Curtis distances (p=0.017). e. Illustration of most enriched phylum on GOH and POH. f. Illustration of most enriched genera on GOH and POH.
Figure 3

The phylogenetic tree of the most enriched ASVs of the microbiome on the TADs. a. The inner circle annotates the genera of the most enriched ASVs. The color reflects the phyla of the specific ASV. The bar plot illustrates the richness of specific ASV in total, and GOH and POH separately.
Figure 4

Differential genera between GOH and POH based on LEfSe test. a. The cladogram depicting the differential microbiome between GOH and POH. b. The bar plot depicting differential genera between GOH and POH. c. Violin plots of the differential genera Veillonella, Streptococcus, Neisseria, Fusobacterium and Porphyromonas.
Figure 5

Correlations between the microbiomes. a. Correlation networks of the abundant genera of microbial communities on the TADs. Spearman correlation coefficients > 0.6 and P-values < 0.05 are shown in the network. Green nodes represent GOH-enriched genera and pink nodes represent POH-enriched genera. Lines in blue between the nodes show positive correlations. Lines in red show negative correlations.
Differential functions based on KEGG database and LEfSe test. a. Differential functional pathways on Level 3. PICRUSt tool was adopted to predict functional roles based on KEGG database. Differentially abundant functional pathways were listed in rows and columns.

**Supplementary Files**

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