Clinical Oral Investigations (2024) 28:373
https://doi.org/10.1007/s00784-024-05763-7

RESEARCH

Microbial colonization in the partially exposed nonabsorbable membrane during alveolar ridge preservation

Silvia Helena Garcia Braz[1] · Mabelle Freitas Monteiro[2] · Edson Ken Matumoto[1] · Mônica Grazieli Corrêa[1] · Renato Corrêa Viana Casarin[2] · Fernanda Vieira Ribeiro[1] · Fabiano Ribeiro Cirano[1] · Marcio Zaffalon Casati[1] · Suzana Peres Pimentel[1]

Received: 21 March 2024 / Accepted: 1 June 2024 / Published online: 14 June 2024
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Abstract

Aim This study evaluated the impact of the partial exposition of the nonabsorbable membrane (dPTFE) on microbial colonization during bone healing.

Materials and Methods Patients indicated for tooth extraction were randomized to *dPTFE group* ($n=22$) - tooth extraction and alveolar ridge preservation (ARP) using an intentionally exposed dPTFE membrane and *USH group* ($n=22$) - tooth extraction and unassisted socket healing. Biofilm samples were collected at the barrier in the dPTFE and on the natural healing site in the USH after 3 and 28 days. Samples from the inner surface of the dPTFE barrier were also collected ($n=13$). The microbiome was evaluated using the Illumina MiSeq system.

Results Beta diversity was different from 3 to 28 days in both groups, and at 28 days, different microbial communities were identified between therapies. The dPTFE was characterized by a higher prevalence and abundance of gram-negative and anaerobic species than USH. Furthermore, the inner surface of the dPTFE membrane was colonized by a different community than the one observed on the outer surface.

Conclusion Intentionally exposed dPTFE membrane modulates microbial colonization in the ARP site, creating a more homogeneous and anaerobic community on the inner and outer surfaces of the membrane.

Clinical relevance DPTFE promoted faster biofilm colonization and enrichment of gram-negative and anaerobes close to the regenerated site in the membrane's inner and outer surfaces. dPTFE membrane can be used exposed to the oral site, but approaches for biofilm control should still be considered.

The study was retrospectively registered at Clinicaltrials.gov (NCT04329351).

Keywords Dental implants · Bone regeneration · Bacteria · Biofilms · Microbiome · 16S

✉ Mabelle Freitas Monteiro
mf.monteiro@yahoo.com.br

Silvia Helena Garcia Braz
shgbraz@gmail.com

Edson Ken Matumoto
edsonkm@yahoo.com.br

Mônica Grazieli Corrêa
monica.grazieli@hotmail.com

Renato Corrêa Viana Casarin
casarinrcv@yahoo.com.br

Fernanda Vieira Ribeiro
ribeirofv@yahoo.com.br

Fabiano Ribeiro Cirano
cirano.fabiano@gmail.com

Marcio Zaffalon Casati
mzcasati@gmail.com

Suzana Peres Pimentel
suppimentel@yahoo.com

[1] Dental Research Division, School of Dentistry, Paulista University, Av. Dr. Bacelar, 1212, 4° andar, Vila Clementino, São Paulo 04026-002, SP, Brazil

[2] Department of Prosthodontics and Periodontics, Piracicaba Dental School, University of Campinas, Av. Limeira, 901, Areião, Piracicaba 13414-903, SP, Brazil

Introduction

Alveolar ridge preservation (ARP) protocols are frequently used after tooth extraction, focusing on reducing bone loss at the extraction site and favoring future rehabilitation with dental implants [1, 2] Several studies have analyzed different ARP approaches and their impact on socket healing [3–5]. The expanded polytetrafluoroethylene (e-PTFE) barriers, one of the most used polymers in ARP, are stable and do not induce immunologic reactions [6] However, when the ePTFE membrane is accidentally exposed to the oral cavity, it can induce bacterial colonization and compromise bone gain [7–9] Given the need to improve the ARP technique, high-density PTFE membranes (dPTFE) started to be studied [10] dPTFE barriers are bioinert, dense, not expanded, and not permeable [11] In addition, they can be an alternative to ePTFE or absorbable membranes, reducing the chances of contamination due to the size of their pores (0.2–0.4 μm) and virtually avoiding the microorganisms' accumulation even when exposed to the oral environment [11–13].

Krauser (1996) examined dPTFE membranes exposed in the oral cavity for 21 days, occasionally showing fibroblast-like cells with no bacteria on the surface, suggesting that this material reduces the risk of infection [14] Posteriorly, Sela and collaborators showed, in vitro, that the adherence and periodontopathogens colonization were higher in absorbable collagen than in dPTFE or ePTFE membranes, showing no difference between the PTFE materials [15] Despite those initial studies, most of the existing literature focused on ePTFE barriers, and few investigated the impact of the dPTFE membranes in regenerative treatments.

Because of these properties, dPTFE membranes have been intentionally exposed during ARP with the argument that this approach can benefit the post-extraction socket and concomitantly minimize bacterial invasion [16–19] A recent clinical trial has evaluated the influence of dPTFE partially exposed on clinical, tomographic, implant-related, and patient-centered outcomes, showing higher keratinized tissue gain for dPTFE but similar bone loss compared to unassisted socket healing (USH) [19] However, there is no clinical evidence from controlled trials of the real impact of this regenerative approach on bacterial colonization on the healing site. The exposed dPTFE membrane can add a new surface for microbial colonization, and there is no clinical evidence that the reduced pore size could avoid microbial invasion and regeneration without the biofilm presence.

Thus, the present study aimed to evaluate, through next-generation sequencing, the influence of intentionally exposed dPTFE membrane on the bacterial colonization profile during the bone repair process.

Materials and methods

Study design

This study is a secondary analysis from a prospective, single-blinded, parallel-arm randomized clinical trial evaluating the impact of dPTFE intentional exposure during ARP. The main results of this study were published [19] and described the clinical, tomographic, immunoenzymatic, implant-related, and patient-centered outcomes after 3 months of ARP. The present publication evaluated the microbiome associated with dPTFE during the initial bone repair process (28 days of dPTFE use). The study was performed according to CONSORT guidelines, registered at Clinicaltrials.gov (NCT04329351), performed in line with the principles of the Declaration of Helsinki, and approved by the ethics committee of Paulista University (2.818.052). Written Informed consent was obtained from all individual participants included in the study.

Population screening

All the individuals were recruited at Paulista University, São Paulo, following the inclusion/exclusion criteria:

Inclusion criteria: Patients 25–70 years old with a maxillary single-rooted tooth indicated for extraction and future rehabilitation with dental implants; exodontia should be indicated due to caries, prosthetic failures, root fractures or endodontic failures. At least three bone walls should be present after tooth extraction.

Exclusion criteria: Pregnancy/lactation, current or former smokers, systemic conditions affecting bone metabolism (e.g., immunologic disorders), chronic use of anti-inflammatory, antibiotic, anti-resorptive, and immunosuppressive medications.

Clinical and radiological exams were performed. Periodontal, endodontic, and restorative needs were made before extraction to provide good oral health previously to the study. The eligible patients were informed of the nature, potential risks, and benefits of participating in the study and signed an informed consent document. Patients were randomly assigned using a computer-generated list into one group after tooth extraction. A complete description of the sample size calculation and randomization process is described in the primary publication [19].

Treatment protocol

All surgeries were performed by the same operator (EKM) using a minimally invasive approach. Patients were randomly designated after tooth extraction to:

dPTFE Group ($n=22$): the dPTFE membrane (CytoplastTi250 Titanium-Reinforced, 12 mm x 24 mm, Osteogenics, Lubbock, TX, USA) was customized and adjusted over the alveolar bone exceeding 3 mm from its border and inserted subperiosteally. Minimal flap reflection was performed to stabilize the membrane, which was intentionally exposed to the oral environment. A cross-mattress dPTFE suture was positioned over the socket to stabilize the membrane (Cytoplast 4 – 0 Suture; CS0618PREM, Osteogenics, Lubbock, TX, USA).

USH Group ($n=22$): tooth extraction with unassisted socket healing (USH). No additional treatment was performed, and the sockets were left to heal spontaneously. The flaps were repositioned, and a suture (Nylon 5.0 Ethicon, Johnson & Johnson, São Paulo, SP, Brazil) was done to stabilize the clot.

The complete treatment protocol and clinical procedures were described in the primary publication [19].

Sutures were removed after 15 days, and the membrane was gently removed under anesthesia after 28 days in the dPTFE group. Temporary adhesive prostheses adapted to the adjacent teeth were installed immediately after surgery. All patients were instructed to rinse with 0.12% chlorhexidine twice daily for four weeks. It was recommended pre-surgery anti-inflammatory therapy (4 mg dexamethasone, single dose) and post-surgery analgesic (500 mg dipyrone sodium, 4/4 hours, 3 days) and antibiotic medication (500 mg amoxicillin 8/8 hours, 7 days).

Microbiological analysis

Biofilm samples were collected in the surgical area 3- and 28-days following tooth extraction. The area was isolated and dried, and the biofilm was removed with sterile cotton swabs from the exposed membrane surface (dPTFE) and the sockets repair region (USH) by the same examiner (SHGB). After its removal, samples from thirteen patients were also collected from the inner surface of the membrane. The samples were stored in microcentrifugation tubes with Tris-EDTA serum at a temperature of -20 °C until the microbiological analysis.

A detailed description of the sequencing protocol and microbial analysis is described in supplemental file 1. The bacterial DNA was isolated using a Qiagen MiniAmp Kit (Valencia, CA) according to the manufacturer's instructions. The hypervariable V3-V4 region of the 16 S rRNA gene was amplified using the primers and the PCR conditions previously described by Klindworth et al. [20]. According to the manufacturer's recommendation, library preparation was performed using the Nextera XT DNA Library Prep Kit. Equimolar DNA concentrations were pooled and sequenced on the Illumina Miseq platform to produce 250 bp paired-end sequences. The sequenced data were deposited and publicly available in the Sequence Read Archive (SRA) database (accession number: PRJNA699468).

Bioinformatic analysis was performed using the QIIME 2 2020.6 [21] After data preprocessing and denoising (q2-DADA2), q2-phylogeny was used to align all amplicon sequence variants (ASVs) and construct the phylogenetic tree. Taxonomy was assigned to ASVs using the q2-feature-classifier trained against the expanded Human Oral Microbiome Database (eHOMD, V15.2) [22] Differences in alpha diversity (Shannon index) were measured using the pairwise differences and the linear-mixed-effects models. The beta diversity metrics (weighted UniFrac and Jaccard distance) were visualized with Principal Coordinate Analysis (PCoA), and the differences between groups were measured with the permanova and permdisp tests (q2-diversity). The impact of time on the analysis was measured with q2-longitudinal pairwise distance. The core microbiome (species presented in at least 50% of the samples from a group) was calculated using the q2-feature-table core features in a collapsed species-level table and visualized with q2-feature-table heatmap and PhyloToAST [23] The differential abundance between ASV was calculated using multinomial regression through q2-Songbird [24], considering the group and the timepoint as dependent variables. The resulting differentials were visualized using log-ratios plots in Qurro [25], and the log-ratio of the ASVs representing 5% of the most important differentials for each group were compared using the two-way ANOVA for repeated measures.

Results

Demographic data

After randomization, two patients were excluded due to severe bone damage after tooth extraction and lack of alveolar bone wall integrity, and they were not microbiologically evaluated. Samples from 20 dPTFE patients and 22 USH patients were analyzed. The primary publication fully describes clinical and demographical data [19].

3,054,892 16 S rRNA gene amplicon raw reads were obtained from the biofilm samples ($n=97$). After using DADA2 for quality control and error modeling, a total of 2,494,958 quality-controlled merged sequences were retained, representing 2768 amplicon sequence variants (ASVs). The sequencing depth was $24{,}949.58 \pm 7520.68$ sequences per sample.

The dPTFE was determinant in modulating the microbial diversity

Three days after surgery, no differences in the alpha (Fig. 1A) and beta diversity (Fig. 1B) were identified between dPTFE and USH. No cluster separation is observed at 3 days (Fig. 1B, Permanova test, $p=0.790$). The follow-up revealed an increase in the alpha diversity at 28 days after surgery for both groups ($p<0.05$). The biofilm maturation was also observed in the beta-diversity (Fig. 1B), with a separation of the 3- and 28-day samples for dPTFE and USH groups (Permanova test, $p=0.001$ and $p=0.002$, respectively). After 28 days of treatment, different beta diversity was identified between groups (Permanova test, $p=0.008$). A more significant impact of time was seen in the microbial community in the dPTFE group compared to USH once the difference between 3 and 28-day pairs was higher in dPTFE (Mann-Whitney test, $p=0.048045$). Furthermore, Jaccard distance between pairs of samples (3 and 28 days for each patient) was used to identify the proportion of features that were not shared between time points, and the dPTFE promoted a higher loss/gain of features during the biofilm growth than the control group (Fig. 1C, Mann-Whitney, $p=0.006286$). It indicates that dPTFE modulates microbiota development and promotes a rapid community shift.

dPTFE exposed to the oral cavity induces changes in the biofilm's core species and regulates the community maturation

Although minor differences were observed in the core microbiome (Fig. 2A) between treatments at 3 days, dPTFE at 3 days has already presented some core species characteristic of dPTFE, such as *Catonella morbi*, *Fusobacterium nucleatum*, and *Campylobacter rectus.* Those gram-negative and anaerobic species are considered secondary colonizers related to a more complex biofilm. At 28 days, there was a more pronounced difference in the core microbiome between groups. The dPTFE presented a bigger core, with 14 exclusive species (such as *Peptostreptococcaceae* and *Ruminococcaceae* families, *Parvimonas micra*, and *Porphyromonas endodontics*) and a more homogeneous community within groups. The relative abundance of core species (Fig. 2B) clearly differentiates the treatments and time points. As seen in the diversity analysis, there was a similarity between groups at the 3-day evaluation and a clear difference between time points in both groups. However, the dPTFE at 28 days demonstrates a unique colonization pattern, reducing the abundance of some species (on the right corner of the graph) and increasing other species (visualized in the middle of the right side of the graph).

dPTFE promoted specific microorganisms' environmental selection, allowing microbial communities differentiation for each treatment

The species describing the most important differentials for dPTFE or USH were illustrated in Fig. 3A and used to compare the groups. They could discriminate the treatments in both time points (Fig. 3B), and features such as *Catonela morbi, Selenomonas genus, Prevotela oris, Peptostreptococcaceae*, and *Ruminococcaceae* families were characteristic of the dPTFE group. In contrast, *Rothia mucilaginosa, Neisseria elongate*, *Streptococcus*, and *Haemophilus* genera represent USH. Interestingly, the selected features representative of dPTFE were more significant at 28 days, although a tendency to increase the log ratios of dPTFE at 3 days is already identified. It suggests that even in the earlier stages of colonization, the dPTFE affects the environment and drives the colonization process.

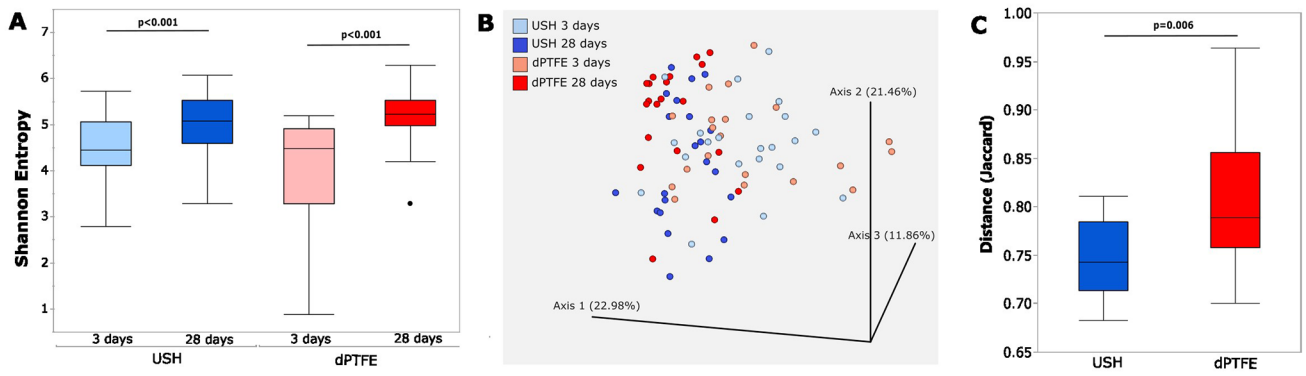

Fig. 1 Diversity metrics. (**A**) Alpha diversity (Shannon Index) in dPTFE and USH groups at each time point. (**B**) Beta diversity: PCoA of the Weighted Unifrac distance for both groups at each time point. (**C**) Beta diversity: pairwise differences (Jaccard distance) between 3 and 28 days for each patient in dPTFE and USH groups, describing the proportion of features that were not shared (lost/gained) between time points

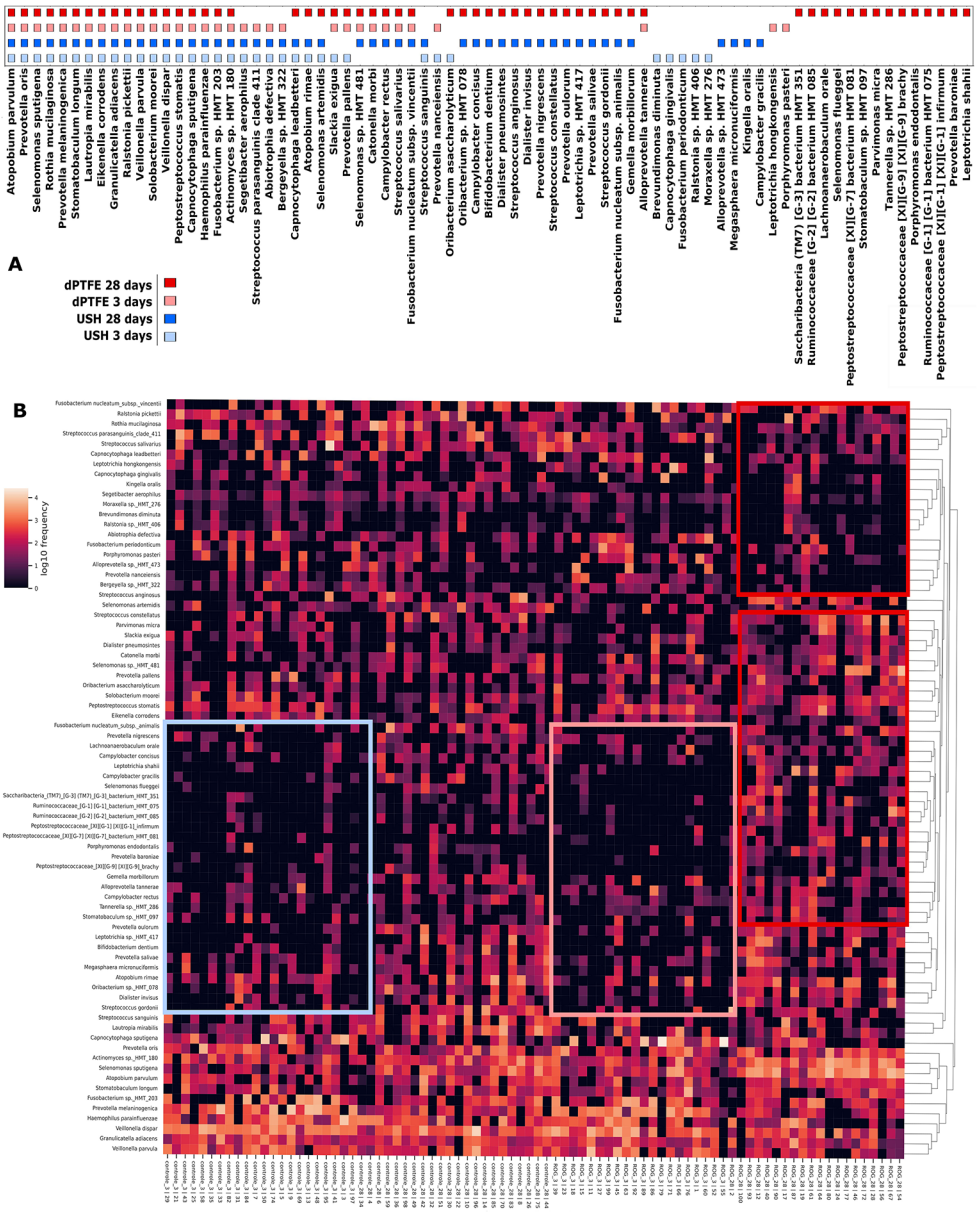

Fig. 2 Changes in the core species during the biofilm maturation in dPTFE and USH groups. (**A**) Core species (species present in at least 50% of the subjects of a group) in dPTFE and USH groups for each time point. (**B**) Heatmap of the relative abundance (log10 frequency) of the core species in the biofilm of dPTFE and USH groups at 3 and 28 days

The dPTFE did not avoid microbial colonization on the inner surface of the barrier, and a unique and mature microbial community was formed in contact with the ARP site

The dPTFE inner surface at 28 days presented a lower microbial diversity (Fig. 4A) than the outer surface (Friedman and a posthoc test, $p = 0.028$), and no differences to the dPTFE 3 days (Friedman and a posthoc test, $p = 0.71$). However, the 3 groups presented different beta diversities in the pairwise comparisons (PERMANOVA test, 3 days vs. inner 28 days – q-value = 0.018; 3 days vs. outer 28 days – q-value = 0.001; inner 28 days vs. outer 28 days – q-value = 0.001). Most of the core microbiome was similar between the outer and inner surface at 28 days (Fig. 4C); however, the inner surface presented an exclusive core composed of *Peptostreptococcaceae* ssp., *Filifactor alocis*, *Treponema socranskii*, *Fretibacterium fastidiosus*, *Prevotella* ssp., while the *Streptococcus* ssp., *Selenomonas* ssp., *Leptotrichia* ssp., and *Ruminococcaceae* ssp. were the exclusive core of the outer surface. In addition, the differential abundance analysis demonstrated that species from those genera could discriminate the microbial community of the inner and outer surface of the dPTFE (Fig. 4D).

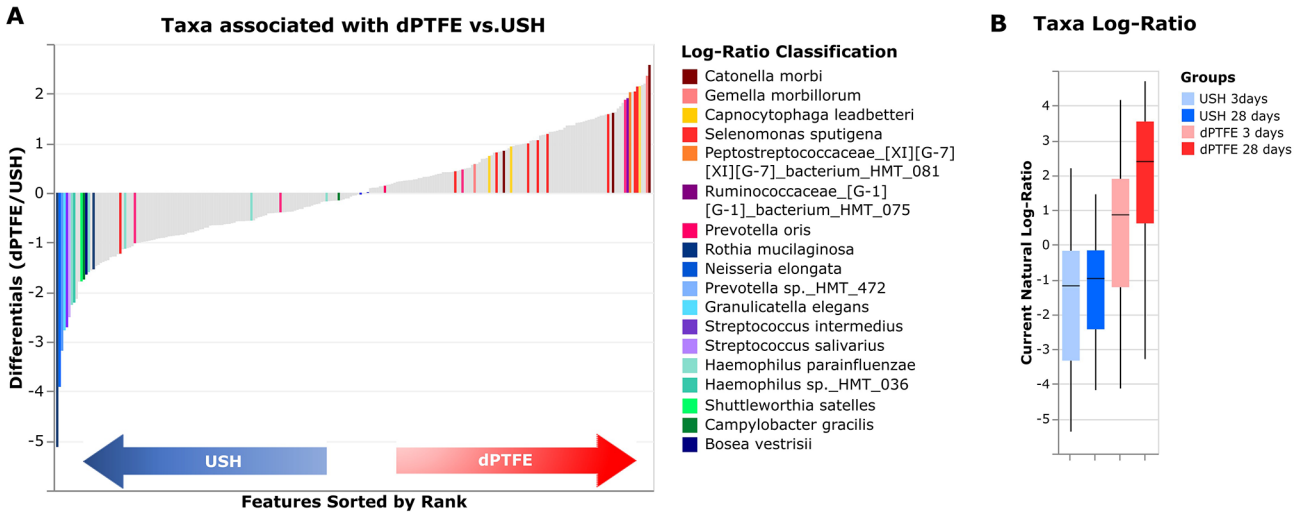

Fig. 3 Differential abundance results of the species described in the top 5% AVS differentials. (**A**) Rank plot of the most discriminative differential. The negative rank values are the most discriminative of the USH group, and the positive differentials are the most discriminative of the dPTFE community. The species described in the legend represents the top 5% of ASV's differentials discriminative of dPTFE and the top 5% differentials for the USH group. (**B**) Log-ratio of the differentials by individual from species selected in 3 A in each group and time point

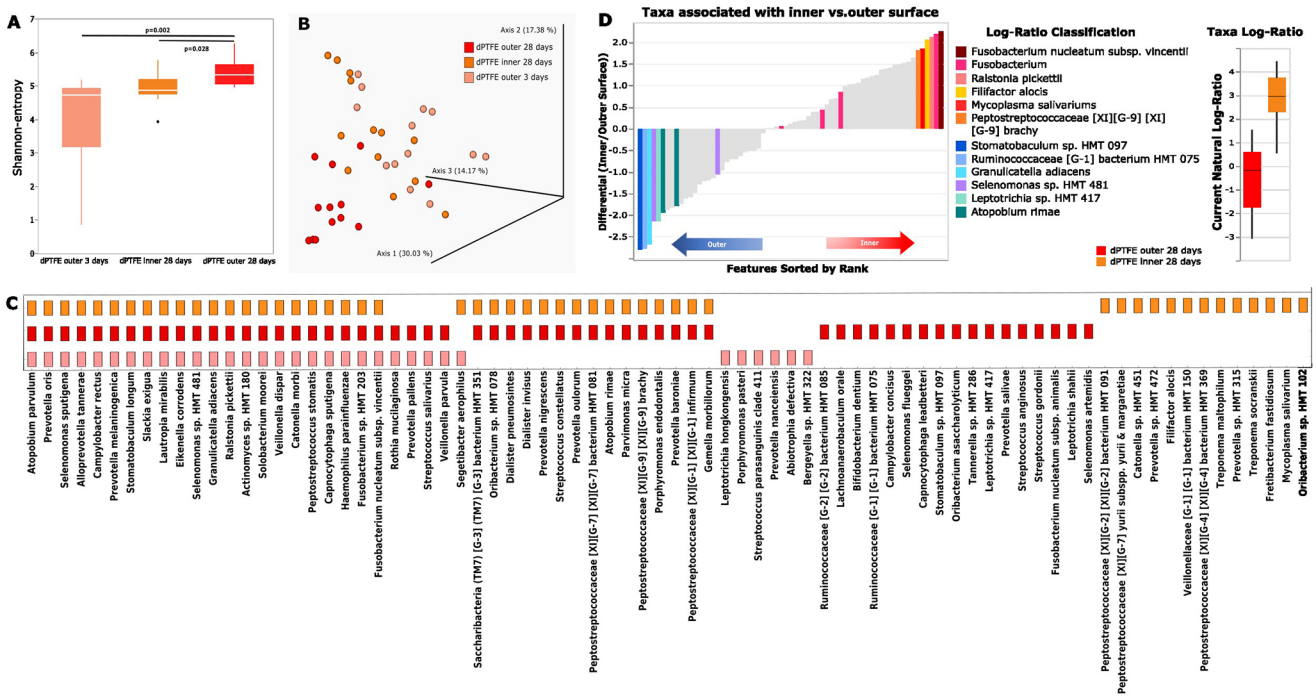

Fig. 4 Characterization of the microbiome colonizing the inner surface of the dPTFE barrier. (**A**) Alpha diversity (Shannon Index) in dPTFE at 3 days, the inner and outer surfaces at 28 days. (**B**) Beta diversity: PCoA of the Weighted Unifrac distances for dPTFE at 3 days, the inner and the outer surfaces at 28 days. (**C**) Core species (species present in at least 50% of the subjects of a group) in dPTFE at 3 days, inner surface, and outer surface at 28 days. (**D**) Differential abundance between the membrane's inner and outer surfaces. The rank plot demonstrates the most discriminative differential, where the negative rank values are the most discriminative of the outer surface, and the positive differentials are the most discriminative of the inner surface at 28 days. The species described in the legend represents the top 10% of ASV's differentials discriminative of each surface. Log-ratio of the taxa identified as the higher differentials to discriminate the barrier surface

Discussion

Using the dPTFE barrier intentionally exposed to the oral environment is a common approach to optimize bone gain in ARP. However, little is known about how this treatment can modulate the colonization in ARP sites during bone healing. The present study evaluated the initial colonization using a microbiome analysis and verified that the intentionally exposed dPTFE membrane drives microbial colonization in a regenerated area, modulating diversity and favoring a more homogeneous and anaerobic community after 28 days of ARP. Furthermore, the dPTFE did not avoid microbial colonization in the membrane's inner surface, which was initially advocated for this membrane due to the reduced size of its pores, but this was not confirmed before in clinical trials.

Unlike the other membrane types, the dPTFE membrane does not require primary wound closure because of the pores' size and structure, promoting the maintenance of the keratinized tissue position and reducing bone loss after tooth extraction [26] Besides, dPTFE intentionally exposed to an oral environment facilitates membrane removal without any other surgical intervention [27], supporting its clinical benefits for ARP. However, although the biofilm formation on the dPTFE membrane has already been demonstrated in in vitro and clinical studies [10, 28–30], to the best of our knowledge, this is the first study describing the ecological shift and the microbial succession in intentionally exposed dPTFE membrane. The membrane exposure to the oral cavity was demonstrated to be an impactful microbiome modulator during healing.

According to the ecological plaque hypothesis, the environment is crucial for the oral site's colonization [31] Thus, alterations in the ecosystem condition, such as the oxygen tension, pH, nutrient supply, and host response, would potentially modulate the colonization and growth of some species. The dPTFE membrane is a rigid and non-shedding surface that favors oral biofilm formation when exposed to the mouth compared to healing tissue. The dPTFE membrane's potential for forming a structured, mature, thick biofilm has already been described in in vitro studies [10, 30] Furthermore, the transition between the tissue margin and the membrane can also favor biofilm protection by reducing the oxygen tension and offering nutrients from the inflammatory process in that region. It can promote biofilm growth and stability and drive community maturation.

The membrane's ecologic pressure on the biofilm formation was demonstrated in the present study, and alterations in the diversity metrics and the core microbiome and a faster new member acquisition were identified. After 3 days, dPTFE had a mild but significant effect on some species' proportions and the core microbiome. The natural microbial succession on biofilm formation occurred in both groups with increased alfa diversity, a change in beta diversity, acquisition of new community members, and increasing relative abundance. However, dPTFE intensified this process. The proportion of species gained/lost over time was significantly higher in the dPTFE, and a different pattern in the relative abundance of the core microbiome was established.

This study also showed that colonization in USH was characterized by *Rothia mucilaginosa, Streptococcus*, and *Haemophilus* genera, for example. Those were aerobic features and representative of colonization's initial stages, described as early colonizers in the tooth [32] Thus, despite the microbial succession identified between 3 and 28 days in the USH, a higher presence of early colonizers and a less complex biofilm on the surface still define this community, where the soft tissue is yet to be formed.

On the other hand, within 28 days, dPTFE presented a unique microbial fingerprint characterized by a more homogenous community. Species such as *Catonella morbi, Gemella morbillorum*, and the genera *Capnocytophaga, Selenomonas*, and *Ruminococcaceae* were highly descriptive of the dPTFE community. Although those members represent a commensal microbiota, they usually depend on early colonizers and more mature biofilm to grow and become dominant in the community, a process facilitated by the membrane's non-shedding surface and the biofilm stability. Additionally, all of those were anaerobic species, suggesting that the membrane creates an environment with lower tension of O_2. The impact of oxygen is even more evident when the community of the inner surface is compared to the membrane's outer surface. Anaerobic species such as *Fusobacterium nucleatum, Filifactor alocis, and Mycoplasma salivariums* were prevalent and in higher abundance on the inner surface, and some species from the genera *Treponema, Fretibacterium, Peptostreptococcaceae*, and *Veillonellaceae* were exclusive from the inner surface core microbiome, highlighting the anaerobiosis of this environment. The oxygen presence is one of the major players in the community definition [33], and more complex and older oral biofilms were usually dependent or more tolerant to an anaerobic environment [34].

Important aspects inherent to the treatment and prosthetic rehabilitation can affect membrane colonization. All patients used temporary adhesive prostheses until the implant placement. It is unclear if provisional prostheses would promote "physical protection" for biofilm removal in the dPTFE, favoring a more complex biofilm after 28 days. Furthermore, the patients used chlorhexidine rinse the whole time when dPTFE was exposed. Using chlorhexidine rinse during healing can also efficiently reduce bacterial adhesion and retard biofilm accumulation on barrier surfaces [35, 36] However, a temporary prosthesis may have compromised

the chlorhexidine access and contact with the operated areas. On the other hand, it could be speculated that USH sites would present tissue desquamation during the repair process, avoiding an un-disturbing biofilm and preventing a more mature biofilm formation.

This investigation supports that the dPTFE procedure impacts the initial microbial colonization in the barrier surface and tissue margins in regenerated sites. However, the primary concern about those findings is if the microbial colonization associated with the dPTFE, especially below the barrier at the inner surface, would present clinical consequences. A previous study described that the ePTFE membrane infiltration with bacterial cells would provide a superior risk for bacterial colonization through the barrier in early exposures, which would promote a reduction in bone gain after periodontal regeneration [37] Sela et al. (1999) showed that while ePTFE and dPTFE present comparable bacteria adherence properties, the low porosity of dPTFE avoids bacterial adherence and infiltration, preventing infectious processes in the regenerated site [15] Even though it was suggested that dPTFE membranes avoid bacterial penetration into the membrane structure [27], a recent study has demonstrated similar bacterial counts and a structured biofilm in the inner and outer surface of dPTFE removed from surgical sited after 28 days [28], corroborating the present study.

Meanwhile, despite the microbial colonization in the inner surface, no patient presented complications or infections related to the ARP. However, it should be taken into consideration that all patients used chlorhexidine for the whole study period, which could also slow down the biofilm formation, and it is not clear if not using the antiseptic could change this clinical scenario of no infection. Another point to be considered is the time using the exposed membrane. The membrane was maintained for 28 days in the ARP site, as the company recommended, and it is not clear if a longer dPTFE exposition and increase in microbial colonization over time could also interfere with this scenario of no infection.

If the colonization of the dPTFE inner surface impacts the bone gain of the regenerative sites, it is hard to confirm with this study design; however, associating microbiological results with the clinical and tomographic data of the study [19] can allow some speculation. This clinical trial's tomographic results demonstrated that dPTFE did not reduce horizontal, vertical, and volumetric bone loss compared to USH [19] Higher bone volume should be expected in ARP procedures compared to USH, and the similarity between therapies can be related to different aspects. It is known that the presence of bone grafts and anatomical conformation of the defects significantly impact the clinical outcomes, and the non-use of graft materials could explain this result, for example. However, based on the membrane microbial colonization observed in the present study, it can be suggested that the microbiome formed because of membrane exposure impacts clinical outcomes. Nevertheless, the study design and the evaluation times (microbiological results evaluated after 28 days of ARP and tomographic results evaluated after 3 months) did not allow a direct correlation between variables and the confirmation that the microbiome impacted bone loss and soft tissue formation. Thus, additional studies are required to confirm this proposition, and microbiological outcomes could be included in future studies of ARP to understand the real impact of membrane colonization on bone repair during the healing process.

Furthermore, the long-term results and the stability of the initial biofilm formation after membrane removal are also unclear. It is possible that, after membrane removal and reduction of the dPTFE ecological pressure, the biofilm returns to the expected colonization profile without producing clinical interference in the regenerated sites. However, this confirmation also needs additional studies.

In conclusion, the present study demonstrated that intentionally exposed dPTFE membrane modulates microbial colonization in the ARP site, promoting rapid biofilm maturation and establishing a more homogeneous and anaerobic community after 28 days of dPTFE. Additionally, the dPTFE did not avoid microbial colonization in the membrane's inner surface close to the ARP site.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00784-024-05763-7.

Acknowledgements This study was supported by the São Paulo Research Foundation (FAPESP) (grant #2017/19834-0). Some analyses presented here were performed using the LaBIS-Cloud service at Laboratório de Biologia Integrativa e Sistêmica (LaBIS - UNICAMP). The LaBIS-Cloud is financed by the FAPESP Process 11/00417-3 and 15/50612-8.

Author contributions FVR contributed to the founding acquisition; FVR, MZC, FRC, and SPP contributed to the work conception and design. SHGB, FRC, and EKM contributed to data acquisition; MFM, MGC, and RCVC contributed to data analysis; MFM and SPP drafted the manuscript; and all authors critically revised and approved the final manuscript.

Funding This study was supported by the São Paulo Research Foundation (FAPESP) (grant #2017/19834-0). Some analyses presented here were performed using the LaBIS-Cloud service at Laboratório de Biologia Integrativa e Sistêmica (LaBIS - UNICAMP). The LaBIS-Cloud is financed by the FAPESP Process 11/00417-3 and 15/50612-8.

Data availability The sequenced data were deposited and publicly available in the Sequence Read Archive (SRA) database (accession number: PRJNA699468).

Declarations

Ethical approval The study was performed in line with the principles of the Declaration of Helsinki and approved by the Ethics Committee of Paulista University (2.818.052). Informed consent was obtained from all individual participants included in the study. The study was performed according to CONSORT guidelines and registered at Clinicaltrials.gov (NCT04329351).

Competing interests The authors declare no competing interests.

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