Early host–microbe interaction in a peri-implant oral mucosa-biofilm model

Carina Mikolai¹ | Nadine Kommerein¹ | Alexandra Ingendoh-Tsakmakidis² | Andreas Winkel¹ | Christine S. Falk² | Meike Stiesch¹

¹Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Hannover, Germany
²Institute of Transplant Immunology, Hannover Medical School, Hannover, Germany

Correspondence
Carina Mikolai, Department of Prosthetic Dentistry and Biomedical Materials Science, Stadtfelddamm 34, 30625 Hannover, Germany.
Email: mikolai.carina@mh-hannover.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: STI 184/6-1;
Niedersächsisches Ministerium für Wissenschaft und Kultur, Grant/Award Number: 74ZN1574

Abstract
The host-microbe relationship is pivotal for oral health as well as for peri-implant diseases. Peri-implant mucosa and commensal biofilm play important roles in the maintenance of host-microbe homeostasis, but little is known about how they interact. We have therefore investigated the early host-microbe interaction between commensal multispecies biofilm (Streptococcus oralis, Actinomyces naeslundii, Veillonella dispar, Porphyromonas gingivalis) and organotypic peri-implant mucosa using our three-dimensional model. After 24 hr, biofilms induced weak inflammatory reaction in the peri-implant mucosa by upregulation of five genes related to immune response and increased secretion of IL-6 and CCL20. Biofilm volume was reduced which might be explained by secretion of β-Defensins-1, -2, and CCL20. The specific tissue reaction without intrinsic overreaction might contribute to intact mucosa. Thus, a relationship similar to homeostasis and oral health was established within the first 24 hr.

In contrast, the mucosa was damaged and the bacterial distribution was altered after 48 hr. These were accompanied by an enhanced immune response with upregulation of additional inflammatory-related genes and increased cytokine secretion. Thus, the homeostasis-like relationship was disrupted. Such profound knowledge of the host-microbe interaction at the peri-implant site may provide the basis to improve strategies for prevention and therapy of peri-implant diseases.

KEYWORDS
biofilms, dental implants, host–microbe interactions, innate immunity, organotypic oral mucosa, peri-implant infections

1 | INTRODUCTION

The oral microbiome consists of several hundred different bacterial species, which are able to form complex multispecies biofilms on oral surfaces (Kolenbrander, Palmer, Periasamy, & Jakubovics, 2010; Zaura, Keijser, Huse, & Crielard, 2009). The early biofilm mainly consists of colonisers typical of oral health (i.e., Streptococcus spp., Actinomyces spp., Veillonella spp.), together with low levels of pathogenic bacteria (i.e., Porphyromonas gingivalis) (Hajishengallis, 2015; Jenkinson & Lamont, 2005). During oral health, a symbiotic relationship exists between the microorganisms and the host (Meyle & Chapple, 2015). The oral mucosa is the first barrier against bacteria (Groeger & Meyle, 2015) and plays an important role in homeostasis -together with commensal bacteria, which are able to induce an early innate
immune response, which is protective (Darveau, 2010). This controlled 
immune response is necessary to maintain oral health (Darveau, 2010; 
Hajishengallis, 2015; Ingendoh-Tsakmakidiset al., 2019). However, lit-
tle is known about the initiation and maintenance of homeostasis in 
the early interaction between commensal biofilm and mucosa 
(Meyle & Chapple, 2015). Various factors (e.g., immunodeficiency, 
environmental factors, keystone pathogens) are able to induce dys-
regulation of the host-microbe homeostasis (Lamont & 
Hajishengallis, 2015). This dysbiosis is accompanied by an increased 
inflammatory reaction and a shift in the microbiome, which can lead to 
oral diseases such as peri-implantitis(Berglundh et al., 2018; Meyle 
& Chapple, 2015). Peri-implantitis is highly prevalent, progresses rapidly 
and is difficult to treat (Belbasakis, 2014; Dreyer et al., 2018). It would 
therefore be desirable to know more about host-microbe homeostasis 
at the implant site in oral health, as this could help to improve strat-
egies for the prevention and therapy of peri-implant diseases. 
Therefore, our aim was to investigate the interaction between an early 
commensal multispecies biofilm and peri-implant mucosa, using our 
3D peri-implantmucosa-biofilm model (Ingendoh-Tsakmakidis-
et al., 2019), in order to get new insights into the early interaction 
between hosts and microbes.

2 | MATERIALS AND METHODS

2.1 | Model of the peri-implant mucosa

The peri-implant mucosa model was constructed as previously 
described (Ingendoh-Tsakmakidiset al., 2019). Briefly, human gingival 
fibroblasts (HGFs) (1,210,412, Provitro GmbH) were embedded in a 
collagen type-1 hydrogel and a HGF-colonised titanium disk (3 mm 
diameter, 2.3 mm height, grade 4, machined surface) was integrated. 
Immortalised human oral keratinocytes (OKF6/TERT-2) (Dickson 
et al., 2000) were seeded on top of the HGF-collagen gel. Models 
were raised to an air-liquid interface, in order to stimulate epithelial 
differentiation and stratification.

2.2 | Multispecies biofilm formation

Streptococcus oralis (ATCC 9811, American Type Culture Collection), 
Actinomyces naeslundii (DSM 43013, German Collection of Microor-
organisms and Cell Cultures), Veillonella dispar (DSM 20735) and 
Porphyromonas gingivalis (DSM 20709) were cultured at 37°C under 
aerobic conditions (80% N2, 10% H2, 10% CO2) in brain heart infu-
sion medium (BHI, Oxoid), supplemented with 10 μg/ml vitamin K. 
The commensal multispecies biofilms were formed as previously 
described (Kommerein et al., 2017). Briefly, equal volumes of the bac-
terial cultures were mixed in BHI/vitamin K to achieve a final optical 
density (600 nm) of 0.01 for each species. The multispecies biofilms 
were grown on glass cover slips (18 mm diameter, thickness 1, Thermo 
Scientific Menzel) in 6-well plates for 48 hr under anaerobic conditions 
(80% N2, 10% H2, 10% CO2) at 37°C.

2.3 | Co-culture of the peri-implant mucosa with 
the multispecies biofilm

After the assembly of the peri-implant mucosa models and the forma-
tion of multispecies biofilms, both were washed separately with 
phosphate-buffered saline (PBS). The multispecies biofilms were 
placed on spacers and on the integrated titanium disk of the peri-
implant mucosa model. The same setting without mucosa was used to 
cultivate the controls of multispecies biofilm. The control mucosa 
models were exposed to a glass cover slip without biofilm. All samples 
were cultured after being submerged in co-culture medium (3:1 
DMEM (P04-03591, Pan-Biotech) and Ham’s F-12 (P04-14559, Pan-
Biotech), 5 μg/ml insulin, 0.4 μg/ml hydrocortisone, 2 × 10−11 M 
5-triiodo-L-thyronine, 1.8 × 10−5 M adenine, 5 μg/ml transferrin, 
10−10 M cholera toxin, 2 mM L-glutamine, 10% v/v FBS, 10% v/v 
BHI/vitamin K) for 24 or 48 hr at 37°C in a humidified 5% CO2 
atmosphere. After 24 hr, the medium was replaced once.

2.4 | Quantitative and qualitative biofilm analysis

After co-culture, the multispecies biofilms were washed once with PBS. 
They were then stained—either with the LIVE/DEAD BacLight Bacterial 
Viability Kit (Life Technologies) or by using fluorescence in situ hybridiza-
tion (FISH) (Kommerein et al., 2017; Kommerein, Doll, Stumpp, & 
Stiesch, 2018). For FISH, the biofilms were fixed and subsequently per-
meabilised for 10 min with 1 μg/ml lysozyme. Hybridization was per-
formed with 4 μM of each 16S rRNA probe (Eurogentec), as listed in 
Appendix Table S1. Both live/dead and FISH-stained biofilms were 
quantified using live/dead staining. The co-localised part was 
measured as both live and dead biofilm. The volume proportions of each bacterial 
species in the multispecies biofilm were quantified using FISH-stained 
biofilms.

2.5 | Histology of the peri-implant mucosa

The histological analysis of the peri-implant mucosa was performed as pre-
viously described (Ingendoh-Tsakmakidiset al., 2019). Briefly, the tissues 
were embedded in Technovit 9,100 and ground to 
22–36 μm slides. Finally, the slides were stained according to van Gieson.

2.6 | RNA extraction and microarray data analysis

RNA extraction and microarray data analysis were performed as previ-
ously described (Ingendoh-Tsakmakidiset al., 2019) and stored in
detail in the GEO database (GSE136274). Briefly, total cellular RNA was isolated by using the RNeasy Mini Kit (Qiagen) with On-column DNase digestion according to the manufacturer's protocol. The quality and quantity of total RNA was determined with the Bioanalyzer 2.100 (Agilent). Synthesis of Cy3-labelled cRNA was performed with the Quick Amp Labeling Kit, One Colour (Agilent), using 500 ng total RNA. The One-Colour Microarray-Based Gene Expression Analysis Protocol V5.7 (Agilent) was applied for Cy3-cRNA fragmentation, hybridization, and washing. Cy3-cRNA (1.650 ng) was hybridised on the refined 0266652QM_RUCG_HomoSapiens microarray (34,127 genes). Slides were scanned on the Agilent Micro Array Scanner G2565CA. Extracted data (Feature Extraction Software V10.7.3.1) were imported into Omics Explorer (software v3.5, Qlucore) under default import settings. Note that one additional sample was imported in parallel to the same Omics Explorer project. Accordingly, calculated baseline and baseline transformed values were influenced by this sample that is not contained in the final data set. For each time point, the co-culture group was compared to the control using the Student's t test (log2 ratio > 2 and p < .05) in the Omics Explorer. Pathway analysis was performed by DAVID (Database for Annotation, Visualisation and Integrated Discovery) (Huang da, da Huang, Sherman, & Lempicki, 2009a; Huang da, da Huang, Sherman, & Lempicki, 2009b) for the upregulated and downregulated gene lists.

2.7 | Quantification of cytokines and human β-Defensins

Cytokine and human β-Defensin (hBD) levels were measured in the collected supernatants. Cytokines CXCL1, IL-10, IL-1β, IL-6, CXCL8, CCL2, CCL20, and TNF-α were quantified using a Human Chemokine Bio-Plex kit (Bio-Rad). These proteins were measured by the Luminex-based multiplex technique according to the manufacturer's instructions. Concentrations were calculated by the Bio-Plex Manager 6.0 using the standard curve with five-parameter logistic (5-PL) regression curves. Cytokine CXCL2, and hBD-1, -2, and -3 were measured using enzyme-linked immunosorbent assays (ELISAs). The ELISA kits were purchased from PeproTech and performed according to the manufacturer's protocol. The concentrations of CXCL2, and hBD-1 to -3 were calculated using a four-parameter logistic (4-PL) equation resulting from the standard curve.

2.8 | Statistical analysis

The statistical analysis was performed using Prism 8 (GraphPad). All the results were analysed using the two-way analysis of variance (ANOVA) with Bonferroni correction. Statistical differences were considered significant at p < .05. The number of individual experiments is stated in the figure legends.

3 | RESULTS

3.1 | Reduction of biofilm volume after peri-implant mucosa exposure

The commensal multispecies biofilms were cultivated with the peri-implant mucosa models or in the same setting without mucosa as control for 24 or 48 hr in a humidified 5% CO2 atmosphere. The biofilm volume was determined by live/dead staining. Exposure to the mucosa significantly reduced the biofilm volume to a similar level after 24 and 48 hr (Figure 1b), which indicates that the biofilm volume was reduced within the first 24 hr. However, the viable part of the biofilm was significantly increased after 48 hr of co-cultivation (Figure 1c).

3.2 | Microbial shift in the multispecies biofilm after 48 hr peri-implant mucosa exposure

The bacterial distribution in the multispecies biofilms was determined by FISH staining. After 24 hr, S. oralis volume dominated the control biofilms, followed by A. naeslundii, V. dispar, and P. gingivalis (Figure 2b). This order did not change after further 24 hr, but there was a significant reduction in the proportion of S. oralis and a significant increase in the proportion of A. naeslundii in the 48 hr control biofilms. Exposure to the mucosa had no impact on bacterial distribution after 24 hr. However, after 48 hr there was a significantly higher volume proportion of S. oralis and V. dispar and a significantly lower volume proportion of A. naeslundii than in the 48 hr control biofilms (Figure 2b).

3.3 | Damage and detachment of the peri-implant mucosa after 48 hr biofilm exposure

After 24 or 48 hr exposure to the biofilm, the impact on the peri-implant mucosa morphology was investigated histologically. Twenty four hours exposure had no visible effect on the peri-implant mucosa (Figure 3a). However, after 48 hr biofilm challenge, the mucosa was slightly damaged and began to detach from the implant (Figure 3a). Bacterial colonisation on the epithelium was detected after 24 and 48 hr (Figure 3b). In contrast to the sporadic colonisation after 24 hr, bacterial colonisation was increased and spread out over the whole epithelium after 48 hr.

3.4 | Increased transcriptional response of the peri-implant mucosa after 48 hr biofilm exposure

The transcriptional activity of the peri-implant mucosa was measured by microarrays. After 24 hr, only five genes were differentially expressed in the peri-implant mucosa exposed to biofilm—in comparison to the unexposed mucosa—and all of these were upregulated.
4.5 | Increased pro-inflammatory cytokine secretion after 48 hr biofilm exposure

The hBD, cytokine and chemokine levels in the supernatants were determined by ELISA or a Luminex-based multiplex assay (Figure 4). Biofilm exposure significantly altered cytokine and chemokine secretion. After 24 hr, IL-6 and CCL20 secretions were increased compared to control, whereas IL-1β, TNF-α, and CCL20 secretions were higher after 48 hr. The CXCL2 level was decreased compared to control at both time points. The constitutive CXCL1, CXCL8, CCL2, hBD-1, and hBD-2 secretion was not enhanced by addition of the biofilm independently from time. hBD-3 and IL-10 secretion was below the detection limit of both ELISA and Luminex-based multiplex assays (data not shown).
Thus, commensal biofilms induce a modest early innate immune response that keeps the tissue in an activated state to protect against pathogens, without intrinsic overreaction of the immune system but with the potential to recruit immune cells (Darveau, 2010). This results in a balanced and controlled immune response and contributes to the maintenance of tissue integrity and oral health (Darveau, 2010; Shang et al., 2018).

The peri-implant mucosa secreted CCL20, hBD-1, and -2, all of which possess antimicrobial activities (Hans & Madaan Hans, 2014; Yang et al., 2003). Both antimicrobial peptides hBD-1 and -2 are expressed in healthy gingival tissue (Dale et al., 2001). hBD-1 is constitutively expressed, whereas hBD-2 can be induced by microbes, except by the Streptococcus cluster (Hans & Madaan Hans, 2014; Langfeldt et al., 2014). hBD-2 was not increased in this study, which...
could be explained by the dominance of S. oralis in our biofilm, which belongs to the Streptococcus cluster. The gene expression and secretion of CCL20 were induced in the peri-implant mucosa after 24 hr exposure to the commensal biofilm. The protective antimicrobial response of the tissue might explain the reduction in the biofilm volume in this study. The control of commensal biofilm overgrowth contributes to oral health (Hans & Madaan Hans, 2014). Biofilm volume decreased without changes in live/dead or in the distribution of bacterial species, which still corresponded to the native early plaque (Kommerein et al., 2017). A stable microbial community is associated with oral health (Kilian et al., 2016). Taken together, in the first phase of biofilm exposure to the peri-implant mucosa, a symbiotic relationship was created between bacteria and the host that was similar to the homeostasis in oral health.

In contrast, 48 hr biofilm exposure to the peri-implant mucosa gave rise to a different reaction from both tissue and biofilm.

### FIGURE 3

Histological sections of the peri-implant mucosa after exposure to either multispecies biofilm or co-culture medium without bacteria for 24 or 48 hr. (a) Implant-mucosa interface is shown. The mucosa was intact in both controls and after 24 hr biofilm exposure. After 48 hr biofilm exposure, the mucosa was detached from the implant and the epithelium was damaged. (b) Bacterial colonisation after 24 or 48 hr. Arrows indicate the bacteria. The ground sections were stained according to van Gieson. Representative pictures of four peri-implant mucosa models for each condition.

### TABLE 1

Enriched and relevant pathways of regulated genes in the peri-implant mucosa after exposure to the multispecies biofilm for 24 or 48 hr.

| Upregulated pathways                        | 24 hr p  | Genes     | 48 hr p  | Genes     |
|---------------------------------------------|----------|-----------|----------|-----------|
| Cytokine-cytokine receptor interaction     | 4.36 × 10⁻⁵ | TNF, CCL20, CXCL2, LTB | 4.89 × 10⁻⁵ | CSF3, TNF, CCL20, LTB, CXCL10 |
| TNF signalling pathway                      | 7.12 × 10⁻⁴ | TNF, CCL20, CXCL2 | 1.87 × 10⁻⁶ | ICAM1, TNF, CCL20, TNFAIP3, CXCL10 |
| NOD-like receptor signalling pathway       | 2.42 × 10⁻² | TNF, CXCL2 | 5.56 × 10⁻² | TNF, TNFAIP3 |
| NF-kappa B signalling pathway              | 3.75 × 10⁻² | TNF, LTB | 6.59 × 10⁻⁵ | ICAM1, TNF, TNFAIP3, LTB |
| Chemokine signalling pathway               | 7.90 × 10⁻² | CCL20, CXCL2 | | |
| RIG-I-like receptor signalling pathway     | | | 6.91 × 10⁻² | TNF, CXCL10 |

| Downregulated pathways                      | 24 hr p  | Genes     | 48 hr p  | Genes     |
|---------------------------------------------|----------|-----------|----------|-----------|
| Protein digestion and absorption            | 1.61 × 10⁻³ | COL13A1, COL6A3, SLCA1, COL5A1, COL10A1 | | |
| MAPK signalling pathway                     | 1.49 × 10⁻² | MEF2C, RASGRP3, HSPA6, PDGFRB, CACNA2D3, FGF2 | | |
| ECM-receptor interaction                    | 1.38 × 10⁻² | LAMA4, COL6A3, ITGA4, COL5A1 | | |
| PI3K-Akt signalling pathway                 | 4.80 × 10⁻² | LAMA4, COL6A3, PDGFRB, ITGA4, FGF2, COL5A1 | | |
| Focal adhesion                              | 3.08 × 10⁻² | LAMA4, COL6A3, PDGFRB, ITGA4, COL5A1 | | |

Note: The pathways with empty table fields were not enriched at this time point. Data from three to four peri-implant mucosa models were used for each condition.
Histological results showed that the epithelial barrier was disrupted and the mucosa was detached from the implant, as is characteristic of peri-implant diseases (Valente & Andreana, 2016). In response to the biofilm, the peri-implant mucosa downregulated genes related to focal adhesions, which are important for the epithelial barrier (Handfield, Baker, & Lamont, 2008) and its attachment to titanium (Pendegrass et al., 2015). Hence, this downregulation might contribute to the epithelial disruption at the implant surface. In addition, the observed enhanced bacterial colonisation and epithelial barrier damage might be promoted by *P. gingivalis*-induced downregulation of the PI3K-Akt signalling pathway and genes related to cell adhesion. *P. gingivalis*, which was a viable part of the biofilm, is able to attenuate the PI3K-Akt signalling pathway (Nakayama, Inoue, Naito, Nakayama, & Ohara, 2015) and to disrupt cell–cell junctions at the levels of gene expression and protein content (Abe-Yutori, Chikazawa, Shibasaki, & Murakami, 2017; Katz, Sambandam, Wu, Michalek, & Balkovetz, 2000). Both promote its colonisation and invasion of mucosal tissue. This epithelium penetration and damage is an important step in the pathogenesis of oral diseases (Groeger, Doman, Chakraborty, & Meyle, 2010). Consequently, the effect on the

---

**FIGURE 4** Cytokine, chemokine and hBD secretion by peri-implant mucosa after exposure to either multispecies biofilm or co-culture medium without bacteria for 24 or 48 hr. The cytokines IL-6, IL-1β, TNF-α, and the chemokines CCL20, CXCL8, CCL2, and CXCL1 were measured by the Luminex-based multiplex technology and hBD1, hBD2, and CXCL2 by ELISA. The Tukey box plots represent the measured data points (18 or 24) of 6 to 8 peri-implant mucosa models for each condition. BF, biofilm; PIM, peri-implant mucosa. *p < .05; **p < .01
peri-implant mucosa indicated that host-microbe homeostasis was disrupted after 48 hr.

After 48 hr, the pro-inflammatory response of the organotypic mucosa was enhanced compared to 24 hr, by additional upregulation of genes related to inflammation and by elevated secretion of CCL20, IL-1β, and TNF-α. Several clinical studies have found that IL-1β levels were significantly higher in patients with periodontitis compared to healthy patients and that they correlated significantly with clinical parameters of periodontitis (Jaedicke et al., 2016). Moreover, excessive production of IL-1β and TNF-α can lead to tissue destruction and loss of attachment of connective tissue (Graves & Cochran, 2003). Consequently, the enhanced and uncontrolled pro-inflammatory response of the peri-implant mucosa leads to the development of mucosal inflammation with tissue destruction (Meyle & Chapple, 2015). These were further signs of disruption of the host-microbe homeostasis after 48 hr.

Bacterial distribution was altered in the biofilm after 48 hr exposure to the peri-implant mucosa. This provides an additional sign of homeostasis disruption, as the relative proportion and diversity of species varies during the development of oral diseases (Kilian et al., 2016). The alteration in bacterial distribution, with the increase in the proportion of *V. dispar*, was probably caused by the release of antimicrobial peptides or tissue breakdown products from the peri-implant mucosa, and/or the presence of *P. gingivalis*, because all have been shown to influence microbial composition (Hajishengallis, 2014; Hajishengallis et al., 2011; Langfeldt et al., 2014). *V. dispar* is an early coloniser and associated with oral health (Avila, Ojcius, & Yilmaz, 2009). An increase of this species in dysbiosis has not yet been described. However, there is some evidence that this species is potentially pathogenic. Veillonella-derived lipopolysaccharide (LPS) induced a toll-like receptor 4 (TLR4)-dependent host cell response (Matera et al., 2009), which is not induced by commensals (McCure & Massari, 2014). TLR signalling can lead to inflammation (McCure & Massari, 2014). Thus, *V. dispar* could play a greater role in the pathogenesis of peri-implant diseases than previously assumed.

There are several explanations of how the initially commensal multispecies biofilm suddenly disrupts the relationship similar to host-microbe homeostasis. According to the keystone pathogen hypothesis (Hajishengallis, Darveau, & Curtis, 2012), *P. gingivalis*, which was present in the multispecies biofilm, can induce a disruption of the homeostasis. It is known that this species, even at very low-colonisation level, is able to induce inflammatory destruction in the presence of commensal bacteria and to remodel the microbial composition (Hajishengallis et al., 2011), as underlined by our results. A further explanation, why the commensal biofilm triggered disruption of homeostasis could be the lack of immune cells in our model. In the in vivo host–microbe interaction, immune cells have an important role in detection of pathogen components as well as in homeostasis (Darveau, 2010). Neutrophils play an important role in the initial reaction in the oral cavity and form a wall between the biofilm and epithelium (Pöllänen, Laine, Ilahi, & Uitto, 2012). The lack of neutrophils through disease or chemical induction invariably causes periodontitis (Darveau, 2010). These clinical situations together with our observations suggest that the induced early innate immune response of the mucosal non-immune compartment is not sufficient to protect the tissue without the support of immune cells. Consequently, a commensal biofilm could cause tissue destruction in the absence of immune cells.

In conclusion, this study demonstrated that an early commensal multispecies biofilm induced a protective pro-inflammatory response in the peri-implant mucosa within the first 24 hr, thus, maintaining host-microbe homeostasis and oral health. However, after further 24 hr incubation, the relationship similar to host-microbe homeostasis was disrupted. Our study suggested that various factors (*V. dispar*, *P. gingivalis*, immune cells) could be involved in the disruption or maintenance of homeostasis. Future investigations will expand the understanding of the pathogenesis of peri-implantitis and help to develop new strategies to prevent and treat peri-implant diseases, as should eventually lead to improved implants.

ACKNOWLEDGMENTS

Microarray data were generated and the 026652QM_RCUG_HomoSapiens microarray was developed by the Research Core Unit Genomics at Hannover Medical School. We would like to thank Dr. Oliver Dittrich-Breiholz and Heike Schneider for support and advice in their use. Ground sections were performed at HIK Hannover and the spacers for the co-cultures were designed by Richard Werth. We would like to thank Ronja Hagemeier, Charlotte Kreuzkamp, Jana Keil and Kerstin Beushausen for their technical assistance.

This work was supported by the DFG [STI 184/6–1] and the R2N Project [74ZN1574], which is funded by the Federal State of Lower Saxony.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Carina Mikolai, Nadine Kommerein, Alexandra Ingendoh-Tsakmakidis, Andreas Winkel, and Meike Stiesch designed experiments; Carina Mikolai and Christine S. Falk performed experiments; Carina Mikolai, Nadine Kommerein, Alexandra Ingendoh-Tsakmakidis, Andreas Winkel, and Christine S. Falk analysed the data; Carina Mikolai wrote the manuscript; Carina Mikolai, Nadine Kommerein, Alexandra Ingendoh-Tsakmakidis, Andreas Winkel, Christine S. Falk, and Meike Stiesch proofread the manuscript.

ORCID

Carina Mikolai https://orcid.org/0000-0002-8816-7766
Alexandra Ingendoh-Tsakmakidis https://orcid.org/0000-0002-4665-8022

REFERENCES

Abe-Yutori, M., Chizakawa, T., Shibasaki, K., & Murakami, S. (2017). Decreased expression of E-cadherin by porphyromonas gingivalis-lipopolysaccharide attenuates epithelial barrier function. *Journal of Periodontal Research*, 52(1), 42–50. https://doi.org/10.1111/jre.12367
gingival epithelial cell adhesion in vitro. European Cells & Materials, 29, 237–249.

Pöllänen, M. T., Laine, M. A., Ihalin, R., & Uitto, V.-J. (2012). Host-bacteria crosstalk at the dentogingival junction. International Journal of Dentistry, 2012, 1-14. http://dx.doi.org/10.1155/2012/821383.

Shang, L., Deng, D., Buskermolen, J. K., Janus, M. M., Krom, B. P., Roffel, S., ... Gibbs, S. (2018). Multi-species oral biofilm promotes reconstructed human gingiva epithelial barrier function. Scientific Reports, 8(1). https://doi.org/10.1038/s41598-018-34390-y

Valente, N. A., & Andreana, S. (2016). Peri-implant disease: What we know and what we need to know. Journal of Periodontal & Implant Science, 46(3), 136–151. https://doi.org/10.5051/jpis.2016.46.3.136

Yang, D., Chen, Q., Hoover, D. M., Staley, P., Tucker, K. D., Lubkowski, J., & Oppenheim, J. J. (2003). Many chemokines including CCL20/MIP-3alpha display antimicrobial activity. Journal of Leukocyte Biology, 74(3), 448–455.

Zaura, E., Keijser, B. J., Huse, S. M., & Crielaard, W. (2009). Defining the healthy “core microbiome” of oral microbial communities. BMC Microbiology, 9. https://doi.org/10.1186/1471-2180-9-259

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.