Local and Systemic Effects of *Porphyromonas gingivalis* Infection

Subjects: Dentistry, Oral Surgery & Medicine

Contributor: William A. Chen, Yuetan Dou, Hansel M. Fletcher, Danilo S. Boskovic

Porphyromonas gingivalis, a gram-negative anaerobe, is a leading etiological agent in periodontitis. This infectious pathogen can induce a dysbiotic, proinflammatory state within the oral cavity by disrupting commensal interactions between the host and oral microbiota. It is advantageous for *P. gingivalis* to avoid complete host immunosuppression, as inflammation-induced tissue damage provides essential nutrients necessary for robust bacterial proliferation. When it gains access to circulation, *P. gingivalis* is able to infect a variety of cells, further triggering a variety of inflammatory and hemostatic responses. Among these is the activation of platelets and neutrophils. Neutrophil responses to *P. gingivalis*, particularly in the presence of activated platelets, include the release of strongly thrombogenic extracellular DNA traps (NETs). Consequently, this pathogen has the capacity not only to promote localized tissue destructiion of the gums but may also trigger some thromboinflammatory processes.

Keywords: dentistry ; inflammation ; neutrophils ; periodontitis ; periodontology ; platelets ; Porphyromonas gingivalis

1. Overview

Porphyromonas gingivalis is a black-pigmented, gram-negative bacterium that primarily colonizes the subgingival tissues in the oral cavity. This asaccharolytic anaerobe can adapt to and survive in the low oxygen tension conditions characteristic of periodontal pockets [1]. However, growth rates under microaerophilic conditions are not significantly altered from those at anaerobic conditions, suggesting that *P. gingivalis* can tolerate microenvironments with low oxygen [2]. Hemin or heme and vitamin K can be used as growth nutrients [3][4]. However, *P. gingivalis* also metabolizes amino acids (AAs) and peptides for energy and as a supply of carbon [5].

Over 700 bacterial species of diverse microbial flora are estimated to inhabit the oral cavity [6]. New culture-independent and culture-dependent molecular techniques have been developed to help characterize microbial communities. Culture-independent approaches include techniques such as next-generation sequencing (NGS) technologies, such as shotgun metagenomics sequencing, allowing researchers to investigate populations of oral bacteria [7]. Deoxyribonucleic acid (DNA) is extracted from the oral microbiome and fragmented prior to sequencing [8]. Then metagenomic analysis helps to highlight the genomic characteristics and potential functions of oral microbiota [9]. Similarly, meta-transcriptomic analyses of ribonucleic acid (RNA) help to assess gene expression in mixed bacterial populations of the oral cavity [10]. Such techniques have been used to investigate interactions of *P. gingivalis* with various other bacterial species and evaluate its effects on the microbial community within the biofilm environment [11][12].

The new culture-dependent techniques employ a variety of media prior to analysis using sensitive mass spectrometric and sequencing techniques, such as matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS) and 16S ribosomal RNA (rRNA) sequencing, to identify bacterial species [13][14]. Future studies can involve such culturomic approaches to characterize particular roles of specific bacteria, such as *P. gingivalis* during the colonization of periodontal tissues and the pathogenesis of periodontitis [15].

The subgingival biofilm, within periodontal pockets, comprises over 300 different species [16]. In this context, *P. gingivalis* is considered to be a late colonizer, often co-aggregating at the top layer with initial and secondary colonizers [17][18]. While most oral microbes are seen as commensal, some, including *P. gingivalis*, are recognized as opportunistic or keystone pathogens [19].

2. *P. gingivalis* Induced Periodontitis

Periodontitis is a proinflammatory state mediated by a pathogenic infection within subgingival tissues. The pathophysiology of periodontitis includes a chronic inflammatory environment that may ultimately progress to a breakdown

of gingival tissues, including periodontal ligament and destruction of supporting structures for teeth [20]. Gradual loss of gingival epithelial attachment to the tooth enamel surface creates deep periodontal pockets that enable further accumulation of biofilm [21].

Removal of the oral biofilm is key to reducing the tissue destruction associated with periodontitis. Scaling and root planing (SRP) remains the gold standard for non-surgical therapy in patients [22]. However, bacterial re-colonization continues to be a limitation of SRP treatment [23]. Other therapeutic approaches are being investigated, including the use of biotics (prebiotics, probiotics, paraprobiotics, lysates, and post-biotics) and various natural compounds [24][25]. Further investigations into such adjunct therapies may provide additional options for controlling microbial biofilm characteristics and modifying clinical outcomes of oral infections, including those with *P. gingivalis*.

Contributing factors to the formation of oral biofilm include increased opportunistic bacterial colonization, periodontitis, dental prosthetics, poor oral hygiene, and smoking [26][27][28]. Specifically, implants may be associated with increased biofilm formation, inflammation, and periodontitis [29]. In such circumstances, supportive periodontal therapy and preventative oral hygiene practice can enhance the success rate of dental prostheses [30][31][32]. Additionally, in order to control microbial growth, new approaches are being explored, including a variety of nanotechnologies [33][34][35].

Biofilm formation comprises cell-to-cell interactions among multiple bacterial species. Early colonizers of the tooth surface include gram-positive anaerobic bacteria such as *Actinomyces* (*A. oris*), *Streptococcus* (*S. gordonii* and *S. mutans*), or *Veillonella* (*V. denticariosi* and *V. parvula*) [36][37]. The abundance of *Actinomyces* sp. and *Streptococcus* sp., based on meta-transcriptome analyses of human supragingival dental biofilm, was 3–12% and 12–19%, respectively [38]. Following their attachment to the pellicle-coated surface of teeth, initial colonizers can facilitate interactions with late colonizers, such as *P. gingivalis*. The surface-expressed polypeptides of *S. gordonii*, Streptococcal surface protein A (SspA), and SspB interact with Mfa1, a protein component of the *P. gingivalis* fimbriae [39][40]. Mfa1 binds to the SspB adherence region (BAR), a discrete region on SspB [41].

Secondary colonizers, such as *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum*, can interact with early colonizers [42]. Subsequently, late colonizers can bind to these secondary colonizers. High interactions between *F. nucleatum* and *P. gingivalis* are observed in coaggregation assays [43]. *P. gingivalis* demonstrated reduced integration into biofilms formed by mutant *F. nucleatum* strains deficient in the outer membrane proteins, fibroblast activation protein 2 (Fap2), and arginine (R)-inhibitable adhesin (RadD) [43].

Interactions between *P. gingivalis* and *F. nucleatum* involve a galactoside moiety and a lectin-like adhesin (FomA), respectively [44][45][46]. Furthermore, the CPS and LPS isolated from *P. gingivalis PK 1924* (serotype K5) can bind to *F. nucleatum* [47]. Consequently, the role of these ‘bridge’ bacteria is to mediate the coaggregation of early and late colonizers [48].

Together, dental biofilm encompasses a diverse bacterial community of over 300 species [16]. Keystone pathogens, such as *P. gingivalis*, transform the biofilm microbiota into a dysbiotic community, which undermines the host immune response and exploits the inflammatory responses to infection. Biofilm buildup propagates persistent chemokine and cytokine production, which is associated with bacterially induced-inflammation of gingival tissues [49]. Ultimately, the diseased pathological state of the periodontium is characterized by irreversible tissue destruction and alveolar bone loss.

3. Pathogen Mediated Dysbiosis

During biofilm formation, *P. gingivalis*, as a late colonizer that adheres to earlier colonizers, is identified as a keystone pathogen implicated in the pathogenesis and progression of periodontitis [18][50]. The polymicrobial dysbiosis model implies that a synergistic equilibrium exists between host gingival tissue and the microbial community [51]. Under physiologic conditions, the oral microbiota comprises heterotypic microbes residing in a controlled symbiotic environment. Host inflammatory and immune responses regulate excessive bacterial proliferation and neutralize overt bacterial pathogenicity [52]. Ordinarily, such homeostasis between the host and the commensal microbiota helps to maintain a balanced state of periodontal health [53]. However, during periodontitis, infectious microbes, such as *P. gingivalis*, disrupt this homeostatic balance and shift the commensal microbial community to a pathogenic state [50]. Even at low abundance, *P. gingivalis* mediates a reinforcing cycle of periodontal dysbiosis leading to enhanced bacterial pathogenicity [54]. This opportunistic pathogen manipulates host responses, locally attenuating the immune system while avoiding total immunosuppression [55]. Chronic infection promotes a continuing proinflammatory environment, including a prominent role of gingipains in the enhanced destructiveness of *P. gingivalis*. Inflammation and gingipain-mediated degradation of

gingival tissue proteins provide peptides, iron, and other nutrients crucial for bacterial growth and further progression of infection.

Dysbiotic polymicrobial communities characteristically develop increased reliance on the nutrients from the serum-like transudate produced during periodontal inflammation [56]. The adoption of a proteolytic phenotype enables all members of this community to thrive. This is in clear contrast to growth limitations when individual members are tested in isolation. Gingipain expression from *P. gingivalis* contributes to the growth of such a microbial community. However, the expression and release of such proteases in the periodontal pocket also appear to be coordinated via signaling from other community members [56]. This microbially driven feedforward inflammatory loop implies a symbiotic enhancement of the overall virulence potential for progressively faster tissue breakdown and microbial growth [57]. Moreover, interactions of *P. gingivalis* with the host as well as other microbial surfaces, are further aided by the adhesive properties of fimbriae [58]. Consequently, the microbial biofilm becomes difficult to displace, leading to continued invasion and persistent tissue destruction.

In turn, the host tissues respond by upregulating the expression of various genes, including ferric ion binding protein, several proto-oncogenes, an ankryn repeat, and a β-enolase [59]. The host iron-binding protein may compete with the microbial community for its iron requirements. The overexpression of the ankryn repeat is commonly associated with various diseases, such as cancer or cardiovascular disorders [60][61]. Similarly, β-enolase has been associated with metabolism in cancer cells [62]. Together, these trends suggest that chronic inflammation may be associated with increased risks for other diseases, possibly including cancer.

4. *P. gingivalis* and Coagulation

The pathogenicity of *P. gingivalis* can potentially extend beyond the oral cavity. Tissue damage from routine oral hygiene practices or dental procedures may facilitate the entry of the periodontopathogen into the systemic circulation [63]. *P. gingivalis* can trigger the activation of prothrombotic mediators, including platelets, increasing the risk for thrombosis [64]. Concentration-dependent shortening of plasma clotting time is observed when human plasma is incubated with purified RgpA or RgpB [65]. *P. gingivalis* expressed gingipains are cysteine proteases, which can activate plasma serine protease coagulation factors [64]. Purified RgpA proteolytically activates factor IX (FIX), factor X (FX), or prothrombin in a concentration and time-dependent manner [65][66][67]. In contrast, purified RgpB cleavage of inactive zymogens yields minimal to no activated factor IX (FIXa), activated factor X (FXa), or thrombin [65][66][67]. The addition of phospholipids and calcium ions, two contributing clotting cofactors, further enhances the RgpA-mediated activation. Moreover, in the presence of phospholipids and calcium ions, RgpA catalytic efficiency (k_{cat}/K_m) of FIX activation is comparable to that observed for a physiological activator, activated factor VII (FVIIa)-tissue factor (TF) complex [67]. However, RgpA is less efficient at FX or prothrombin activation compared to FVIIa-TF or FXa-activated factor V (FVa) complex, respectively [65] [66]. Several snake venoms contain enzymes known to activate coagulation factors, including FX or prothrombin [68][69]. In this context, RgpA-mediated FX activation is comparable to that of Russell's viper venom [65]. In addition, the prothrombin activation rate by RgpA is higher compared to *Notechis scutulus scutulus* venom but lower compared to venom from *Oxyuranus scutellatus* [66]. Taken together, FIX, FX, and prothrombin activation by RgpA may be contributing factors in thrombin production.

5. The Role of Gingipains in Platelet Function

Bacterial infection is often transient for individuals with a robust immune system. However, serious thrombotic complications can develop from persistent infection, including infective endocarditis and sepsis-associated disseminated intravascular coagulation [70]. A distinct feature is the bacterially mediated platelet activation leading to the formation of intravascular thrombi [71]. *P. gingivalis* interaction with platelets can induce platelet activation and subsequent aggregation [72]. Intracellular calcium mobilization is associated with platelet activation [73]. Consistent with this, if treated with live *P. gingivalis*, isolated platelets undergo intracellular calcium mobilization [72]. However, this does not occur if resting platelets are treated with heat-killed bacteria or with the double (*rgpA* and *rgpB*) gingipain knockout mutant. A single (*kgp*) gingipain mutant did elicit changes in intracellular calcium levels, however, this was significantly lower compared to platelet exposure to the wild-type strain. Similarly, platelet aggregation is observed following the incubation of *P. gingivalis* with isolated platelets. However, platelet aggregation depends on the ratio of platelet/bacteria, consistent with the possibility of either a threshold phenomenon or with multiple competing platelet interactions. In whole blood, platelet expression of CD62P, an adhesion molecule expressed on surfaces of activated platelets, increases following preincubation of high *P. gingivalis* colony-forming units (CFU) with or without subsequent ADP stimulation [74]. Conversely, there is a trend towards higher CD62P expression even in response to low *P. gingivalis* CFU, particularly as preincubation time is extended. This suggests a dose and time dependence for the impact of *P. gingivalis* preincubation on platelet surface CD62P expression.

High levels of *P. gingivalis* may promote an excitable state in platelets that results in rapid activation following subsequent interaction with physiologic agonists. At lower *P. gingivalis* levels, platelet responses may be triggered with prolonged preincubation times. In this context, whole blood from generalized aggressive periodontitis and periodontitis patients is associated with higher platelet activation [75][76]. Moreover, robust platelet aggregation is observed after incubation of *P. gingivalis* with whole blood from patients with the peripheral arterial disease (PAD) [77]. Similarly, agonist-dependent increases in platelet P-selectin expression are observed after systemic *P. gingivalis* infusion into rats [78]. Preincubation of *P. gingivalis* with whole blood also impacts platelet plug formation under shear conditions [79]. Extending *P. gingivalis* preincubation times past 7.5 min significantly reduces the time for platelet plug-mediated aperture occlusion in the Platelet Function Analyzer (PFA-100). Thus, platelet plug formation time in whole blood is affected both by the *P. gingivalis* concentration and by the duration of bacterial preincubation.

Interestingly, a prolongation of the occlusion time can be observed at certain *P. gingivalis* levels below those needed for the occlusion time shortening [79]. This is explainable either (a) by ineffective platelet activation or (b) by alternate platelet activation pathways. During the bacterial preincubation phase, platelets may become activated in response to interaction with *P. gingivalis*. However, the activated platelets may be insufficient to trigger full platelet aggregation. Consequently, the spent activated platelets become refractory to platelet plug formation, leading to a prolonged occlusion time. Alternatively, if *P. gingivalis* is capable of interacting with multiple platelet activation pathways with characteristic interaction affinities, then multiple platelet functions could be triggered in a concentration-dependent manner. As a result, *P. gingivalis* in whole blood may trigger a variety of time-dependent processes, some of which are possibly functionally opposing [79].

Platelets are involved in a variety of ways with leukocyte functions, including those of neutrophils. Platelet-neutrophil interactions are believed to be mediated by an interaction between platelet P-selectin and neutrophil P-selectin glycoprotein ligand-1 (PSGL-1) [80]. Such interaction is enhanced following ADP-mediated platelet activation [74]. Platelet-neutrophil interactions are also enhanced in the presence of *P. gingivalis* in a preincubation time-dependent manner. Moreover, bacterial exposure to whole blood can trigger the neutrophil release of nuclear DNA, also known as neutrophil extracellular traps (NETs). Such release of NETs in response to *P. gingivalis* is known to be at least in part dependent on an interaction between activated platelets and neutrophils [74]. This implies that the interaction of *P. gingivalis* with the various blood cells does not only potentially alter their cell-specific functions in response to this pathogen but can also impact their physiologic cell-cell interactions.

Protease-activated receptors (PARs), members of the GPCR family, are characterized by a unique activation mechanism. The amino terminus of PARs is cleaved to expose an auto-activating tethered ligand that triggers intracellular signal transduction via an internal salt bridge formation [81]. Human platelets express two types of PARs, PAR-1, and PAR-4. These receptors are normally proteolytically activated by the serine protease thrombin as one of the mechanisms of platelet activation [82]. *P. gingivalis* expressed gingipains, however, can also cleave and activate PAR-1 and PAR-4 [83]. RgpA is up to six-fold more efficient in activating PAR-4 compared to thrombin [83]. Thrombin, however, is significantly more efficient at PAR-1 activation compared to either RgpA or RgpB [83]. The particular activation efficiency of PAR-1 by thrombin is likely due to a hirudin-like sequence contained within the exodomain of PAR-1, which binds with high affinity to the anion-binding exosite of thrombin [84]. Furthermore, cytosolic calcium levels are increased following the incubation of isolated platelets with purified RgpA or RgpB. Pretreatment of platelets with an anti–PAR-1 antibody abrogates this effect, supporting the role of arginine gingipain dependent PAR-1 cleavage in platelet calcium activities [83]. Similarly, the treatment of platelets with a protease inhibitor completely abolished this effect [83]. In this context, lower levels of RgpA are required to induce platelet aggregation compared to RgpB, emphasizing its higher efficiency at mediating platelet responses.

However, the proteolytic functions of gingipains are not solely responsible for mediating platelet aggregation. In the presence of *P. gingivalis*, platelet aggregation is observed in platelet-rich plasma (PRP) treated individually or in combination with inhibitors for Rgp or Kgp [85]. This suggests that other bacterial products may also mediate some platelet aggregating effects. Hgp44, an adhesin domain expressed at the C-termini of RgpA and Kgp, plays a role in hemagglutination and hemoglobin binding [86]. Incubating PRP with a mutant *P. gingivalis* strain deficient in adhesin domains only or with a strain deficient in Rgp, Kgp, and adhesin domains does not induce platelet aggregation [85]. However, platelet aggregating potential is restored when a recombinant Hgp44 is preincubated with either mutant strain prior to incubation with PRP. Furthermore, incubating PRP with *P. gingivalis* in the presence of anti-Fc_{γ}RIIa mAb inhibits platelet aggregation [85]. Similarly, platelet aggregation can be somewhat reduced when PRP is incubated with *P. gingivalis* and an anti-glycoprotein (GP) Ibα mAb. However, aggregation of washed platelets, treated with gingipain deficient strains, is restored if anti-*P. gingivalis* immunoglobulin G (IgG) is added [85]. Taken together, *P. gingivalis* can induce platelet aggregation independent of gingipains via pathways that involve contributing roles from Fc_{γ}RIIa, IgG, and GPIbα.

6. Conclusions

Porphyromonas gingivalis is a gram-negative anaerobic opportunistic pathogen that infects the subgingival tissues of the oral cavity. It is a late colonizer that disrupts the relationship between the local commensal microbes and the host. Consequently, *P. gingivalis* is a leading etiological agent in periodontitis. It is advantageous for *P. gingivalis* to avoid complete host immunosuppression, as inflammation-induced tissue damage provides essential nutrients necessary for robust bacterial proliferation. In this context, *P. gingivalis* can gain access to the systemic circulation, where it can promote a prothrombotic state. *P. gingivalis* expresses a number of virulence factors, which aid this pathogen toward infection of a variety of host cells, evasion of detection by the host immune system, subversion of the host immune responses, and activation of several humoral and cellular hemostatic factors.

References

1. Loesche,W.J.; Gusberti, F.; Mettraux, G.; Higgins, T.; Syed, S.; Relationship between Oxygen-Tension and Subgingival Bacterial- Flora in Untreated Human Periodontal Pockets. *Infect. Immun.* **1983**, *42*, 659-667, .
2. Lewis, J.P.; Iyer, D.; Anaya-Bergman, C.; Adaptation of Porphyromonas gingivalis to microaerophilic conditions involves increased consumption of formate and reduced utilization of lactate. *Microbiol. Sgm.* **2009**, *155*, 3758–3774, .
3. Olczak, T.; Simpson,W.; Liu, X.Y.; Genco, C.A.; Iron and heme utilization in Porphyromonas gingivalis. *Fems. Microbiol. Rev.* **2005**, *29*, 119–144, .
4. Wyss, C.; Growth of Porphyromonas gingivalis, Treponema denticola, T. pectinovorum, T. socranskii, and T. vincentii in a chemically defined medium. *J. Clin. Microbiol.* **1992**, *30*, 2225–2229, .
5. Nelson, K.E.; Fleischmann, R.D.; DeBoy, R.T.; Paulsen, I.T.; Fouts, D.E.; Eisen, J.A.; Daugherty, S.C.; Dodson, R.J.; D urkin, A.S.; Gwinn, M.; et al. Complete genome sequence of the oral pathogenic bacterium Porphyromonas gingivalis st rain W83. *J. Bacteriol.* **2003**, *185*, 5591–5601, .
6. Aas, J.A.; Paster, B.J.; Stokes, L.N.; Olsen, I.; Dewhirst, F.E.; Defining the normal bacterial flora of the oral cavity. *J. Cli n. Microbiol.* **2005**, *43*, 5721–5732, .
7. Martellacci, L.; Quaranta, G.; Patini, R.; Isola, G.; Gallenzi, P.; Masucci, L. A.; A Literature Review of Metagenomics and Culturomics of the Peri-implant Microbiome: Current Evidence and Future Perspectives.. *Materials* **2019**, *12*, 3010, .
8. Sedghi, L.; DiMassa, V.; Harrington, A.; Lynch, S.V.; Kapila, Y.L.; The oral microbiome: Role of key organisms and com plex networks in oral health and disease. *Periodontol. 2000* **2021**, *87*, 107–131, .
9. Quince, C.; Walker, A.W.; Simpson, J.T.; Loman, N.J.; Segata, N.; Shotgun metagenomics, from sampling to analysis. *Nat. Biotechnol.* **2017**, *35*, 833–844, .
10. Duran-Pinedo, A.E.; Metatranscriptomic analyses of the oral microbiome. *Periodontol. 2000* **2021**, *85*, 28–45, .
11. Frias-Lopez, J.; Duran-Pinedo, A.; Effect of Periodontal Pathogens on the Metatranscriptome of a Healthy Multispecies Biofilm Model. *J. Bacteriol.* **2012**, *194*, 2082–2095, .
12. Zhang, Y.F.; Shi, W.Y.; Song, Y.Q.; Wang, J.F.; Metatranscriptomic analysis of an in vitro biofilm model reveals strain-sp ecific interactions among multiple bacterial species. *J. Oral. Microbiol.* **2019**, *11*, 1599670, .
13. Lagier, J.C.; Dubourg, G.; Million, M.; Cadoret, F.; Bilen, M.; Fenollar, F.; Levasseur, A.; Rolain, J.M.; Fournier, P.E.; Ra oult, D.; et al. Culturing the human microbiota and culturomics. *Nat. Rev. Microbiol.* **2018**, *16*, 540–550, .
14. Lagier, J.C.; Hugon, P.; Khelaifia, S.; Fournier, P.E.; La Scola, B.; Raoult, D.; The Rebirth of Culture in Microbiology thro ugh the Example of Culturomics To Study Human Gut Microbiota. *Clin. Microbiol. Rev.* **2015**, *28*, 237–264, .
15. Bashiardes, S.; Zilberman-Schapira, G.; Elinav, E.; Use of Metatranscriptomics in Microbiome Research. *Bioinform. Bio l. Insights* **2016**, *10*, 19–25, .
16. Paster, B.J.; Boches, S.K.; Galvin, J.L.; Ericson, R.E.; Lau, C.N.; Levanos, V.A.; Sahasrabudhe, A.; Dewhirst, F.E.; Bact erial diversity in human subgingival plaque. *J. Bacteriol.* **2001**, *183*, 3770–3783, .
17. Kolenbrander, P.E.; Palmer, R.J.; Periasamy, S.; Jakubovics, N.S.; Oral multispecies biofilm development and the key r ole of cell-cell distance. *Nat. Rev. Microbiol.* **2010**, *8*, 471–480, .
18. Zijnge, V.; van Leeuwen, M.B.M.; Degener, J.E.; Abbas, F.; Thurnheer, T.; Gmur, R.; Harmsen, H.J.M.; Oral Biofilm Arch itecture on Natural Teeth. *PLoS ONE* **2010**, *5*, e9321, .
19. Olsen, I.; Lambris, J.D.; Hajishengallis, G.; Porphyromonas gingivalis disturbs host-commensal homeostasis by changi ng complement function. *J. Oral. Microbiol.* **2017**, *9*, 1340085, .

20. Kinane, D.F.; Stathopoulou, P.G.; Papapanou, P.N.; Periodontal diseases. *Nat. Rev. Dis. Primers.* **2017**, *3*, 17038, .

21. Lockhart, P.B.; Bolger, A.F.; Papapanou, P.N.; Osinbowale, O.; Trevisan, M.; Levison, M.E.; Taubert, K.A.; Newburger, J.W.; Gornik, H.L.; Gewitz, M.H.; et al. Periodontal Disease and Atherosclerotic Vascular Disease: Does the Evidence S upport an Independent Association? A Scientific Statement from the American Heart Association. *Circulation* **2012**, *125*, 2520–2544, .

22. Sanz, I.; Alonso, B.; Carasol, M.; Herrera, D.; Sanz, M.; Nonsurgical treatment of periodontitis. *J. Evid. Based. Dent. Pr act.* **2012**, *12*, 76–86, .

23. Berezow, A.B.; Darveau, R.P.; Microbial shift and periodontitis. *Periodontol. 2000* **2011**, *55*, 36–47, .

24. Butera, A.; Gallo, S.; Maiorani, C.; Preda, C.; Chiesa, A.; Esposito, F.; Pascadopoli, M.; Scribante, A.; Management of Gingival Bleeding in Periodontal Patients with Domiciliary Use of Toothpastes Containing Hyaluronic Acid, Lactoferrin, o r Paraprobiotics: A Randomized Controlled Clinical Trial. *Appl. Sci. Basel.* **2021**, *11*, 8586, .

25. Ghasemi, N.; Behnezhad, M.; Asgharzadeh, M.; Zeinalzadeh, E.; Kafil, H.S.; Antibacterial Properties of Aloe vera on Int racanal Medicaments against Enterococcus faecalis Biofilm at Different Stages of Development. *Int. J. Dent.* **2020**, *202 0*, 8855277, .

26. Belibasakis, G.N.; Microbiological and immuno-pathological aspects of peri-implant diseases. *Arch. Oral Biol.* **2014**, *59*, 66–72, .

27. Kumar, P.S.; Matthews, C.R.; Joshi, V.; de Jager, M.; Aspiras, M.; Tobacco Smoking Affects Bacterial Acquisition and C olonization in Oral Biofilms. *Infect. Immun.* **2011**, *79*, 4730–4738, .

28. Marsh, P.D.; Are dental diseases examples of ecological catastrophes?. *Microbiol. Sgm.* **2003**, *149*, 279–294, .

29. Hao, Y.; Huang, X.Y.; Zhou, X.D.; Li, M.Y.; Ren, B.; Peng, X.; Cheng, L.; Influence of Dental Prosthesis and Restorative Materials Interface on Oral Biofilms. *Int. J. Mol. Sci.* **2018**, *19*, 3157, .

30. Cattoni, F.; Tete, G.; D'Orto, B.; Bergamaschi, A.; Polizzi, E.; Gastaldi, G.; Comparison of hygiene levels in metal-ceram ic and stratified zirconia in prosthetic rehabilitation on teeth and implants: A retrospective clinical study of a three-year f ollow-up. *J. Biol. Regul. Homeost. Agents.* **2021**, *35*, 41–49, .

31. Graziani, F.; Karapetsa, D.; Alonso, B.; Herrera, D.; Nonsurgical and surgical treatment of periodontitis: How many opti ons for one disease?. *Periodontol 2000* **2017**, *75*, 152–188, .

32. Merlone, A.; Tete, G.; Cantile, N.; Manacorda, M.; Cattoni, F.; Minimally invasive digital implant-prosthetic procedure in "all on 4" rehabilitation in patients with special needs: A three-year follow-up. *J. Biol. Regul. Homeost. Agents.* **2021**, *35*, 71–85, .

33. Chi, C.; Springer, B.N.; Walemba, E.; Nick, K.E.; Perry, C.C.; Kwon, S.R.; Titanium-oxide nanoparticles and nanofibers used alone or with UV light activation.. *CDA J.* **2019**, *47*, 777–782, .

34. Holden, M.S.; Black, J.; Lewis, A.; Boutrin, M.C.; Walemba, E.; Sabir, T.S.; Boskovic, D.S.; Wilson, A.; Fletcher, H.M.; P erry, C.C.; et al. Antibacterial activity of partially oxidized Ag/Au nanoparticles against the oral pathogen Porphyromona s gingivalis W83. *J. Nanomater.* **2016**, *2016*, 9605906, .

35. Kwon, S.R.; Li, Y.; Walemba, E.M.; Bozhilov, K.N.; Perry, C.; Potential penetration of CTAB-and MUDA-coated gold nan orods into tooth enamel. *J. Contemp. Dent. Pract.* **2020**, *21*, 475–480, .

36. Marcotte, H.; Lavoie, M.C.; Oral microbial ecology and the role of salivary immunoglobulin A. *Microbiol. Mol. Biol. R.* **19 98**, *62*, 71–109, .

37. Mashima, I.; Nakazawa, F.; The influence of oral Veillonella species on biofilms formed by Streptococcus species. *Anae robe* **2014**, *28*, 54–61, .

38. Benitez-Paez, A.; Belda-Ferre, P.; Simon-Soro, A.; Mira, A.; Microbiota diversity and gene expression dynamics in hum an oral biofilms. *BMC Genomics* **2014**, *15*, 311, .

39. Brooks, W.; Demuth, D.R.; Gil, S.; Lamont, R.J.; Identification of a Streptococcus gordonii SspB domain that mediates adhesion to Porphyromonas gingivalis. *Infect. Immun.* **1997**, *65*, 3753–3758, .

40. Lamont, R.J.; El-Sabaeny, A.; Park, Y.; Cook, G.S.; Costerton, J.W.; Demuth, D.R.; Role of the Streptococcus gordonii SspB protein in the development of Porphyromonas gingivalis biofilms on streptococcal substrates. *Microbiol. Sgm.* **20 02**, *148*, 1627–1636, .

41. Park, Y.; Simionato, M.R.; Sekiya, K.; Murakami, Y.; James, D.; Chen,W.B.; Hackett, M.; Yoshimura, F.; Demuth, D.R.; L amont, R.J.; et al. Short fimbriae of Porphyromonas gingivalis and their role in coadhesion with Streptococcus gordonii. *Infect. Immun.* **2005**, *73*, 3983–3989, .

42. Kolenbrander, P.E.; Andersen, R.N.; Blehert, D.S.; Egland, P.G.; Foster, J.S.; Palmer, R.J.; Communication among oral bacteria. *Microbiol. Mol. Biol. R.* **2002**, *66*, 486–505, .

43. Park, J.; Shokeen, B.; Haake, S.K.; Lux, R.; Characterization of Fusobacterium nucleatum ATCC 23726 adhesins involved in strain-specific attachment to Porphyromonas gingivalis. *Int. J. Oral Sci.* **2016**, *8*, 138–144, .

44. Kinder, S.A.; Holt, S.C.; Characterization of Coaggregation between Bacteroides-Gingivalis T22 and Fusobacterium-Nucleatum T18. *Infect. Immun.* **1989**, *57*, 3425–3433, .

45. Kinder, S.A.; Holt, S.C.; Localization of the Fusobacterium-Nucleatum T18 Adhesin Activity Mediating Coaggregation with Porphyromonas-Gingivalis T22. *J. Bacteriol.* **1993**, *175*, 840–850, .

46. Shaniztki, B.; Hurwitz, D.; Smorodinsky, N.; Ganeshkumar, N.; Weiss, E.I.; Identification of a Fusobacterium nucleatum PK1594 galactose-binding adhesin which mediates coaggregation with periopathogenic bacteria and hemagglutination. *Infect. Immun.* **1997**, *65*, 5231–5237, .

47. Rosen, G.; Sela, M.N.; Coaggregation of Porphyromonas gingivalis and Fusobacterium nucleatum PK 1594 is mediated by capsular polysaccharide and lipopolysaccharide. *Fems. Microbiol. Lett.* **2006**, *256*, 304–310, .

48. Hojo, K.; Nagaoka, S.; Ohshima, T.; Maeda, N.; Bacterial Interactions in Dental Biofilm Development.. *J. Dent. Res.* **2009**, *88*, 982–990, .

49. Cekici, A.; Kantarci, A.; Hasturk, H.; Van Dyke, T.E.; Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol. 2000* **2014**, *64*, 57–80, .

50. Hajishengallis, G.; Darveau, R.P.; Curtis, M.A.; The keystone-pathogen hypothesis. *Nat. Rev. Microbiol.* **2012**, *10*, 717–725, .

51. Hajishengallis, G.; Lamont, R.J.; Beyond the red complex and into more complexity: The polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. *Mol. Oral Microbiol.* **2012**, *27*, 409–419, .

52. Lamont, R.J.; Hajishengallis, G.; Polymicrobial synergy and dysbiosis in inflammatory disease. *Trends. Mol. Med.* **2015**, *21*, 172–183, .

53. Hajishengallis, G.; Lamont, R.J.; Polymicrobial communities in periodontal disease: Their quasi-organismal nature and dialogue with the host. *Periodontol. 2000* **2021**, *86*, 210–230, .

54. Darveau, R.P.; Hajishengallis, G.; Curtis, M.A.; Porphyromonas gingivalis as a Potential Community Activist for Disease. *J. Dent. Res.* **2012**, *91*, 816–820, .

55. Hajishengallis, G.; Lamont, R.J.; Breaking bad: Manipulation of the host response by Porphyromonas gingivalis. *Eur. J. Immunol.* **2014**, *44*, 328–338, .

56. Davies, J.R.; Kad, T.; Neilands, J.; Kinnby, B.; Prgomet, Z.; Bengtsson, T.; Khalaf, H.; Svensater, G.; Polymicrobial synergy stimulates Porphyromonas gingivalis survival and gingipain expression in a multi-species subgingival community. *BMC Oral Health* **2021**, *21*, 639, .

57. Lamont, R.J.; Koo, H.; Hajishengallis, G.; The oral microbiota: Dynamic communities and host interactions. *Nat. Rev. Microbiol.* **2018**, *16*, 745–759, .

58. Xu, W.; Zhou, W.; Wang, H.; Liang, S.; Roles of Porphyromonas gingivalis and its virulence factors in periodontitis. *Adv. Protein. Chem. Struct. Biol.* **2020**, *120*, 45–84, .

59. Deng, Z.L.; Szafranski, S.P.; Jarek, M.; Bhuju, S.; Wagner-Dobler, I.; Dysbiosis in chronic periodontitis: Key microbial players and interactions with the human host. *Sci. Rep.* **2017**, *7*, 3703, .

60. Mosavi, L.K.; Cammett, T.J.; Desrosiers, D.C.; Peng, Z.Y.; The ankyrin repeat as molecular architecture for protein recognition. *Protein. Sci.* **2004**, *13*, 1435–1448, .

61. Moulik, M.; Vatta, M.; Witt, S.H.; Arola, A.M.; Murphy, R.T.; McKenna,W.J.; Boriek, A.M.; Oka, K.; Labeit, S.; Bowles, N.E.; et al. ANKRD1, the Gene Encoding Cardiac Ankyrin Repeat Protein, Is a Novel Dilated Cardiomyopathy Gene. *J. Am. Coll. Cardiol.* **2009**, *54*, 325–333, .

62. Kim, A.Y.; Lim, B.; Choi, J.; Kim, J.; The TFG-TEC oncoprotein induces transcriptional activation of the human beta-enolase gene via chromatin modification of the promoter region. *Mol. Carcinog.* **2016**, *55*, 1411–1423, .

63. Forner, L.; Larsen, T.; Kilian, M.; Holmstrup, P.; Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. *J. Clin. Periodontol.* **2006**, *33*, 401–407, .

64. Guo,W.; Wang, P.; Liu, Z.H.; Ye, P.; Analysis of differential expression of tight junction proteins in cultured oral epithelial cells altered by Porphyromonas gingivalis, Porphyromonas gingivalis lipopolysaccharide, and extracellular adenosine triphosphate. *Int. J. Oral Sci.* **2018**, *10*, e8, .

65. Imamura, T.; Potempa, J.; Tanase, S.; Travis, J.; Activation of blood coagulation factor X by arginine-specific cysteine proteinases (gingipain-Rs) from Porphyromonas gingivalis. *J. Biol. Chem.* **1997**, *272*, 16062–16067, .

66. Imamura, T.; Banbula, A.; Pereira, P.J.B.; Travis, J.; Potempa, J.; Activation of human prothrombin by arginine-specific cysteine proteinases (gingipains R) from Porphyromonas gingivalis. *J. Biol. Chem.* **2001**, *276*, 18984–18991, .
67. Imamura, T.; Tanase, S.; Hamamoto, T.; Potempa, J.; Travis, J.; Activation of blood coagulation factor IX by gingipains R, arginine-specific cysteine proteinases from Porphyromonas gingivalis. *Biochem. J.* **2001**, *353*, 325–331, .
68. Kini, R.M.; The intriguing world of prothrombin activators from snake venom. *Toxicon* **2005**, *45*, 1133–1145, .
69. Tans, G.; Rosing, J.; Snake venom activators of factor X: An overview. *Haemostasis* **2001**, *31*, 225–233, .
70. Hamzeh-Cognasse, H.; Damien, P.; Chabert, A.; Pozzetto, B.; Cognasse, F.; Garraud, O.; Platelets and infections—Complex interactions with bacteria. *Front. Immunol.* **2015**, *6*, 82, .
71. Cox, D.; Kerrigan, S.W.; Watson, S.P.; Platelets and the innate immune system: Mechanisms of bacterial-induced platelet activation. *J. Thromb. Haemost.* **2011**, *9*, 1097–1107, .
72. Klarstrom Engstrom, K.; Khalaf, H.; Kalvegren, H.; Bengtsson, T.; The role of Porphyromonas gingivalis gingipains in platelet activation and innate immune modulation. *Mol. Oral Microbiol.* **2015**, *30*, 62–73, .
73. Varga-Szabo, D.; Braun, A.; Nieswandt, B.; Calcium signaling in platelets.. *J. Thromb. Haemost.* **2009**, *7*, 1057–1066, .
74. Chen, W.A.; Fletcher, H.M.; Payne, K.J.; Aka, S.; Thornburg, M.B.; Gheorghe, J.D.; Safi, S.B.; Shavlik, D.; Oyoyo, U.; Boskovic, D.S.; et al. Platelet and neutrophil responses to Porphyromonas gingivalis in human whole blood. *Mol. Oral Microbiol.* **2021**, *36*, 202–213, .
75. Papapanagiotou, D.; Nicu, E.A.; Bizzarro, S.; Gerdes, V.E.A.; Meijers, J.C.; Nieuwland, R.; van der Velden, U.; Loos, B.G.; Periodontitis is associated with platelet activation. *Atherosclerosis* **2009**, *202*, 605–611, .
76. Zhan, Y.L.; Lu, R.F.; Meng, H.X.; Wang, X.; Hou, J.X.; Platelet activation and platelet-leukocyte interaction in generalized aggressive periodontitis. *J. Leukocyte. Biol.* **2016**, *100*, 1155–1166, .
77. Notani, H.; Inoue, Y.; Sugano, N.; Jibiki, M.; Umeda, M.; Izumi, Y.; Whole-blood platelet aggregation by Porphyromonas gingivalis in patients with peripheral arterial disease. *J. Med. Dent. Sci.* **2011**, *58*, 7–14, .
78. Yu, K.M.; Inoue, Y.; Umeda, M.; Terasaki, H.; Chen, Z.Y.; Iwai, T.; The peridontal anaerobe Porphyromonas gingivalis induced platelet activation and increased aggregation in whole blood by rat model. *Thromb. Res.* **2011**, *127*, 418–425, .
79. Chen, W.A.; Fletcher, H.M.; Gheorghe, J.D.; Oyoyo, U.; Boskovic, D.S.; Platelet plug formation in whole blood is enhanced in the presence of Porphyromonas gingivalis.. *Mol. Oral Microbiol.* **2020**, *35*, 251–259, .
80. Etulain, J.; Martinod, K.; Wong, S.L.; Cifuni, S.M.; Schattner, M.; Wagner, D.D.; P-selectin promotes neutrophil extracellular trap formation in mice. *Blood* **2015**, *126*, 242–246, .
81. Gieseler, F.; Ungefroren, H.; Settmacher, U.; Hollenberg, M.D.; Kaufmann, R.; Proteinase-activated receptors (PARs)—Focus on receptor-receptor-interactions and their physiological and pathophysiological impact. *Cell Commun. Signal.* **2013**, *11*, 86, .
82. Kahn, M.L.; Nakanishi-Matsui, M.; Shapiro, M.J.; Ishihara, H.; Coughlin, S.R.; Protease-activated receptors 1 and 4 mediate activation of human platelets by thrombin. *J. Clin. Invest.* **1999**, *103*, 879–887, .
83. Lourbakos, A.; Yuan, Y.P.; Jenkins, A.L.; Travis, J.; Andrade-Gordon, P.; Santulli, R.; Potempa, J.; Pike, R.N.; Activation of protease-activated receptors by gingipains from Porphyromonas gingivalis leads to platelet aggregation: A new trait in microbial pathogenicity. *Blood* **2001**, *97*, 3790–3797, .
84. Vu, T.K.H.; Wheaton, V.I.; Hung, D.T.; Charo, I.; Coughlin, S.R.; Domains Specifying Thrombin-Receptor Interaction. *Nature* **1991**, *353*, 674–677, .
85. Naito, M.; Sakai, E.; Shi, Y.; Ideguchi, H.; Shoji, M.; Ohara, N.; Yamamoto, K.; Nakayama, K.; Porphyromonas gingivalis-induced platelet aggregation in plasma depends on Hgp44 adhesin but not Rgp proteinase. *Mol. Microbiol.* **2006**, *59*, 152–167, .
86. Sakai, E.; Naito, M.; Sato, K.; Hotokezaka, H.; Kadowaki, T.; Kamaguchi, A.; Yamamoto, K.; Okamoto, K.; Nakayama, K.; Construction of recombinant hemagglutinin derived from the gingipain-encoding gene of Porphyromonas gingivalis, identification of its target protein on erythrocytes, and inhibition of hemagglutination by an interdomain regional peptide. *J. Bacteriol.* **2007**, *189*, 3977–3986, .