

## New approaches to combat *Porphyromonas gingivalis* biofilms

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### ABSTRACT

In nature, bacteria predominantly reside in structured, surface-attached communities embedded in a self-produced, extracellular matrix. These so-called biofilms play an important role in the development and pathogenesis of many infections, as they are difficult to eradicate due to their resistance to antimicrobials and host defense mechanisms. This review focusses on the biofilm-forming periodontal bacterium *Porphyromonas gingivalis*. Current knowledge on the virulence mechanisms underlying *P. gingivalis* biofilm formation is presented. In addition, oral infectious diseases in which *P. gingivalis* plays a key role are described, and an overview of conventional and new therapies for combating *P. gingivalis* biofilms is given. More insight into this intriguing pathogen might direct the development of better strategies to combat oral infections.

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### Introduction

Biofilms are aggregates of microorganisms adherent to each other and/or to a surface and encapsulated within a self-produced matrix [1]. These organized communities represent a significant health risk due to their resistance to host defense mechanisms and their decreased susceptibility to conventional antimicrobials [1,2]. Biofilm-mediated resistance has been attributed to impaired penetration of antimicrobials through the matrix, increased expression of drug-resistance genes, and reduced metabolic activity of cells residing in the biofilm [3]. Because of their involvement in >80% of all bacterial infections in humans, biofilms have been the subject of intensive research for many years [4].

The oral cavity provides a habitat for approximately 700 microbial species forming complex and dynamic multispecies biofilms, also referred to as 'dental plaque' [5,6]. The oral Gram-negative anaerobic bacteria *P. gingivalis* is typically a late colonizer of subgingival biofilms and has been correlated with several destructive periodontal diseases, including periodontitis and peri-implantitis [7–9]. *P. gingivalis*' pathogenicity is reflected in an arsenal of virulence factors involved in tissue colonization and destruction, and interference with host defense systems [10,11].

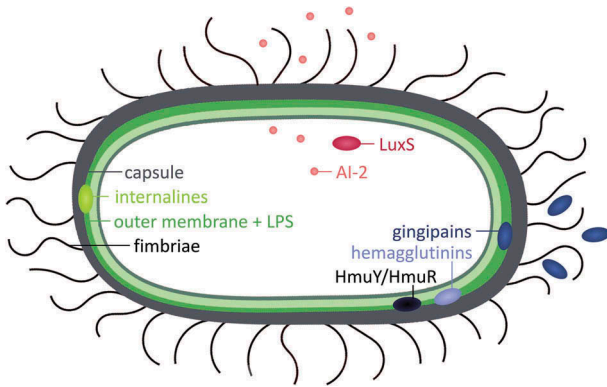
In this review, an overview of the current knowledge on *P. gingivalis* biofilm formation is first presented. Next, biofilm infections in which *P. gingivalis* plays a key role are described, and finally conventional treatment strategies and new approaches to combat *P. gingivalis* biofilms are discussed.

### Biofilm formation by *P. gingivalis*

Biofilm development is a complex, dynamic, multi-stage process [1]. Initially, bacteria adhere to abiotic or biotic surfaces by production of surface appendages (initial adherence). Next, biofilms mature by the development of a three-dimensional structure containing microcolonies in which different species can interact with each other (biofilm maturation). In the last phase, cells disperse from the biofilm, allowing the formation of new biofilms (biofilm dispersal) [1,12]. Novel strategies to treat *P. gingivalis* infections benefit from thorough insight into the virulence mechanisms underlying biofilm formation. However, to date, knowledge on the molecular basis of biofilm formation by *P. gingivalis* is largely fragmentary. Approximately 18% of the *P. gingivalis* genome is differentially expressed when the bacteria is grown as a biofilm, demonstrating the complexity of biofilm development [13]. Below, we describe the involvement of surface structures, quorum sensing, heme uptake, and nutritional interactions in *in vitro* biofilm formation by this pathogen (Figure 1).

### Role of surface structures in biofilm formation

Given the wide variety of substrates to which *P. gingivalis* can attach in the oral cavity (e.g. oral soft tissues, implant materials, and other bacteria), many extracellular structures play a role in mediating specific and stable substrate attachment. Examples include fimbriae, lipopolysaccharides (LPS), internalines, and capsules.



**Figure 1.** Determinants involved in biofilm formation by *Porphyromonas gingivalis*. A schematic representation of the involvement of surface structures (fimbriae, lipopolysaccharides, internalines, and capsules), quorum sensing (LuxS/AI-2), and heme uptake (gingipains, hemagglutinins and HmuY/HmuR) in *in vitro* biofilm formation.

Fimbriae are proteinaceous appendages that are anchored to the outer membrane and play a role in biofilm formation (Figure 2). *P. gingivalis* is known to express two types of fimbriae: long fimbriae, which are composed of FimA subunits, and short fimbriae, which are composed of Mfa1 subunits [14]. Loss of FimA results in reduced adherence to human gingival fibroblasts and epithelial cells, demonstrating that FimA fimbriae play a role in the initial attachment of bacteria to host cells [15,16]. Furthermore, long fimbriae are involved in *P. gingivalis* auto-aggregation [17,18] and *P. gingivalis* co-aggregation with *Actinomyces viscosus* [19], *Treponema denticola* [20], *Streptococcus gordonii* [21], and *Streptococcus oralis* [22]. Of note, mutants deficient in *gtfA*, a putative glycosyltransferase gene (PG0750), are affected in biofilm development. These mutants lack mature FimA fimbriae but have an unchanged *fimA*

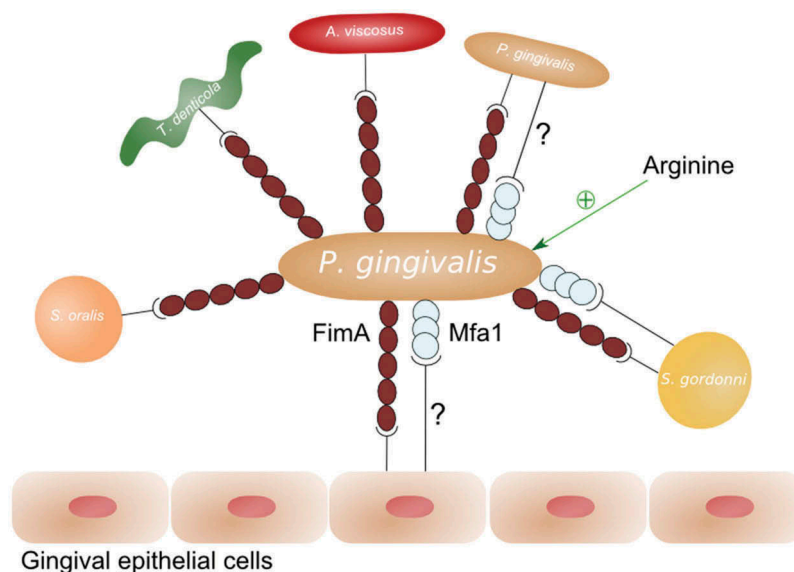
expression, indicating that sugar transfer is involved in fimbriae development [23].

Little is known about the role of Mfa1 in *P. gingivalis* biofilm formation. Short fimbriae are involved in *P. gingivalis* co-aggregation with *S. gordonii* [24]. On the other hand, Mfa1-deficient mutants were reported to display enhanced auto-aggregation [18]. The latter is contradicted by an earlier report showing that short fimbriae are required for *P. gingivalis* auto-aggregation [25]. Supporting these findings, elevated expression of short fimbriae in a ClpXP-deficient strain results in increased *P. gingivalis* biofilm formation [26].

Recent studies have illustrated the importance of extracellular arginine in fimbriae-mediated biofilm formation. *P. gingivalis* is unable to form microcolonies with *Streptococcus cristatus* as a result of a down-regulation of *fimA* expression by streptococcal ArcA, which catalyzes the hydrolysis of arginine to citrulline [27]. Similarly, ArcA from *Streptococcus intermedius* represses FimA and Mfa1 production in *P. gingivalis* [28]. Finally, the addition of arginine promotes *P. gingivalis* biofilm formation [29].

In addition to fimbriae, surface-associated LPS have been shown to mediate *P. gingivalis* biofilm formation. LPS typically consist of three parts: a lipid A moiety that tethers LPS to the outer membrane, a core oligosaccharide, and an O-antigen polysaccharide [30]. The absence of GalE, which is involved in the synthesis of sugar nucleotides in the Leloir pathway, results in shortened LPS O-antigens and significantly increases *P. gingivalis* biofilm formation [31].

Furthermore, surface-attached internalines, which are involved in protein-protein interactions, have been shown to play a role in the initial



**Figure 2.** Overview of interactions of *P. gingivalis* fimbriae FimA and Mfa1 with epithelial cells and other bacteria. A question mark indicates a hitherto unclear effect or interaction.

attachment of the bacteria [32]. Indeed, inactivation of the internalin family protein InlJ reduces *P. gingivalis* monospecies biofilm formation and enhances mixed-species *P. gingivalis*–*S. gordonii* biofilm formation [32].

Last, *P. gingivalis* is known to produce a capsular polysaccharide, which encases the cell surface, thereby masking surface components such as LPS and surface proteins. Interestingly, loss of capsule production positively affects biofilm formation of *P. gingivalis* [33]. On the other hand, it was reported that *P. gingivalis* capsules mediate co-aggregation between *P. gingivalis* and *Fusobacterium nucleatum* [34].

### Role of quorum sensing in biofilm formation

Quorum sensing is a bacterial communication mechanism in which the expression of genes is coordinated through the accumulation of specific signaling molecules [35]. *P. gingivalis* utilizes the LuxS/Autoinducer-2 (AI-2) quorum sensing system [36,37]. *luxS* encodes the AI-2 synthase, which cleaves S-ribosylhomocysteine into 4,5-dihydroxy-2,3-pentanedione (DPD). Subsequently, DPD undergoes spontaneous derivatizations, forming signaling molecule AI-2 [38]. This quorum sensing system has been shown to play a role in interspecies communication of *P. gingivalis*. More specifically, AI-2 was demonstrated to be necessary for the formation of *P. gingivalis*–*S. gordonii* mixed biofilms, and AI-2 produced by *S. gordonii* is able to complement a *luxS* mutation in *P. gingivalis* [39]. Similarly, the biovolume of *Filifactor alocis*–*P. gingivalis* mixed-species biofilms was significantly reduced with a *P. gingivalis luxS*-mutant, indicating a role for AI-2 in the interaction between *P. gingivalis* and *F. alocis* [40]. In addition, it was shown that AI-2 from *Actinobacillus actinomycescomitans* is capable of complementing a *luxS* mutation in *P. gingivalis* [41]. Finally, it was demonstrated that AI-2 from *F. nucleatum* induced both *P. gingivalis* monospecies biofilm formation and *F. nucleatum*–*P. gingivalis* mixed-species biofilm formation [42].

### Role of heme and heme uptake systems in biofilm formation

Iron is an essential growth factor for most bacteria. Unlike many other microorganisms, *P. gingivalis* does not produce siderophores to sequester and transport iron. Instead, the bacterium utilizes specific proteases such as gingipains and surface-associated proteins to acquire iron from host heme [43].

Proteolytic gingipains Kgp and Rgp play an important role in the acquisition of iron by releasing heme from hemoglobin [43]. In addition, it was found that Kgp suppresses *P. gingivalis* auto-aggregation, and Rgp mediates microcolony formation and restrains the

biovolume [18]. Furthermore, gingipains are involved in adherence of *P. gingivalis* to epithelial cells and in co-aggregation of *P. gingivalis* with *T. denticola*, *Prevotella intermedia*, and *S. aureus* [44–46].

Surface-expressed hemagglutinins mediate the acquisition of heme through erythrocyte binding [47]. Furthermore, the heme-binding outer-membrane-associated lipoprotein HmuY and its cognate receptor HmuR are involved in the capture and internalization of heme [48]. Deletion of *hmuY* or the hemagglutinin gene *hagC* results in reduced levels of biofilm, suggesting a role in biofilm formation [49,50]. In addition, the hemin-associated transcriptional regulator Har, which controls the expression of *hmuY* and *mfa1*, was found to be a positive regulator of biofilm formation [51]. This study also demonstrated that heme-limitation *per se* decreases *P. gingivalis* biofilm formation and development [51].

### Role of nutritional interactions in biofilm formation

Nutritional interactions have been described to play a role in the co-existence of *P. gingivalis* and *T. denticola*. A study revealed that *P. gingivalis* produces isobutyric acid, which enhances growth of *T. denticola*, while *T. denticola* produces succinate that positively affects growth of *P. gingivalis* [52]. This may explain the finding that *P. gingivalis* and *T. denticola* show enhanced planktonic and biofilm growth when they are cultured together in comparison to monospecies growth [52,53].

### Treatment of *P. gingivalis*-related infections

*P. gingivalis* is one of the most prevalent bacteria in periodontitis, a chronic inflammatory disease of the oral cavity [8]. This disease is characterized by destruction of the supporting structures of the teeth (i.e. the gingiva, the periodontal ligament, and the alveolar bone) and can eventually lead to loss of teeth [54]. Furthermore, periodontitis has recently been associated with an increased risk for delivery of premature labor and low-birth-weight infants [55]. The prevalence of periodontitis is high, with the moderate form affecting up to 46% and the severe form 8.9% of the US population [56]. Smoking and diabetes are known major risk factors for periodontitis [57].

*P. gingivalis* is also recognized as a keystone pathogen in peri-implantitis, a periodontal disease characterized by inflammation of the hard and soft tissues surrounding dental implants. When left untreated, peri-implantitis can result in loss of the dental implant [9,58]. Studies have reported a considerably high prevalence of peri-implantitis, with estimations ranging from 20% to 56% of the patients, depending on the time frame under investigation [59,60].

## Current strategies

Treatment procedures of *P. gingivalis*-mediated diseases such as periodontitis and peri-implantitis focus on the eradication of oral pathogens at the site of infection, usually by surface debridement procedures followed by adjunctive therapies, including the use of antiseptics or/and antibiotics [61–66].

The antiseptic chlorhexidine has been widely used in dental practice because of its broad-spectrum antimicrobial activity [67]. Local application of chlorhexidine can be done in the form of gels, chips, mouthwashes, or films [68–71]. Despite its widespread use, some limitations have been reported, including brown discoloration of the teeth, alteration in taste, supragingival calculus formation, and, more rarely, oral mucosal erosion and parotid swelling. Additionally, chlorhexidine is characterized by a bitter taste, contributing to patient non-compliance [72–74].

Several antibiotic classes have also been suggested for the treatment of *P. gingivalis*-related infections, including tetracyclines (tetracycline hydrochloride, minocycline, doxycycline), macrolides (erythromycin), lincosamides (clindamycin),  $\beta$ -lactams (ampicillin, amoxicillin), and nitroimidazoles (metronidazole) [64,75–77]. These antibiotics can be administered by either local or systemic routes. Systemically administered antibiotics can penetrate the periodontal tissues and reach deep periodontal pockets via serum. In this way, antibiotics can target oral pathogens that are inaccessible for cleaning instruments or locally applied antibiotics [78]. However, this application route requires good patient compliance, can cause side effects, and can facilitate antibiotic resistance [66,79–81]. Local administration has the advantage that higher therapeutic concentration of antibiotics can be delivered inside the pocket, avoiding some of the side effects of systemic administration.

In the last few years, concerns have been raised about the efficacy of the aforementioned antimicrobials in treatment of oral biofilm-related infections. First, compared with planktonic cells, *P. gingivalis* cells residing in biofilms are less susceptible to antimicrobials such as chlorhexidine, minocycline, metronidazole, amoxicillin, doxycycline, cefuroxime, ampicillin, and ofloxacin [82–87]. More specifically, biofilms can be up to 500 times less sensitive to antibiotics [87]. Second, several studies have examined the antibiotic susceptibility of subgingival microflora isolated from patients suffering from periodontitis and peri-implantitis. This is illustrated by the finding that 74.2% of patients with periodontitis and 71.7% of patients with peri-implantitis harbor pathogens resistant to at least one standard antibiotic [80,81]. A recent study also reported that 25.49%, 23.52%, and 21.56% of the *P. gingivalis* strains isolated from patients with periodontitis are

resistant to amoxicillin, clindamycin, and metronidazole, respectively [88]. Similarly, periodontitis isolates of *P. gingivalis* were demonstrated to be resistant against penicillin, amoxicillin, erythromycin, azithromycin, clindamycin, and tetracycline [89].

The current data concerning biofilm-mediated resistance in dental practice, together with the emerging global threat of antimicrobial resistance, have prompted researchers to search for new antimicrobial agents targeting oral pathogens. Newly identified antibacterial agents that show activity against *P. gingivalis* biofilms are discussed below.

## New antibacterial agents

### Quorum sensing inhibitors

Quorum sensing inhibitors have been presented as promising alternatives for the treatment of biofilm-related infections, as they do not affect growth and thus have a low potential for resistance development [90]. In this respect, quorum sensing inhibitors (5Z)-4-bromo-5-(bromomethylene)-2(5H)-furanone (2 mM) and D-ribose (50 mM) have been shown to reduce both *P. gingivalis* monospecies and *F. nucleatum* and *P. gingivalis* mixed-species biofilm development [42]. Furthermore, these agents are not toxic for human monocytic cells and human gingival fibroblasts at tested concentrations, and do not stimulate production of proinflammatory factors. In addition, these inhibitors remain active against *P. gingivalis* under *in vivo* conditions, making them suitable candidates for further development into anti-*P. gingivalis* drugs [91].

### Antimicrobial peptides

Antimicrobial peptides are widely proposed as a new source of future antibiotics, as they often have broad-spectrum activity and a low tendency for resistance development [92]. An overview of the currently known antimicrobial peptides that show antibiofilm activity against *P. gingivalis* is given in Table 1.

The shortened alanine-substituted peptide AS10, derived from the cathelicidin-related antimicrobial peptide, which was identified in the islets of Langerhans of the murine pancreas, was reported to inhibit *P. gingivalis* biofilm formation [93]. Agents derived from lactoferrin, an iron-binding host defense antimicrobial protein present in saliva and gingival crevicular fluids, also exhibit antibiofilm activity against *P. gingivalis* [94]. In addition, Nal-P-113, which is a  $\beta$ -naphthylalanine-substituted derivative of the saliva protein histatin 5, has an effect on *P. gingivalis* biofilm formation under both *in vitro* and *in vivo* conditions, without significant cytotoxicity [95]. Furthermore, a recent study demonstrated that the newly designed peptide Pac-525 has the ability to kill bacteria within *P. gingivalis* biofilms formed on



**Table 1.** Overview of antimicrobial peptides effective against *Porphyromonas gingivalis* biofilm formation.

Antimicrobial peptide	Active concentration	Reference
Cathelicidin-related antimicrobial peptide (CRAMP)	20.3 µM	[93]
Lactoferrin derivatives	8 µg/mL	[94]
Nal-P-113	6.25 µg/mL	[95]
Pac-525	0.5 µg/mL	[96]
BAR	1.3 µM	[97]

titanium surfaces at a concentration that does not exert cytotoxic effects against eukaryotic cells [96].

The adhesion of *P. gingivalis* to primary colonizing bacteria such as *S. gordonii* has been recognized as an important step in the initial formation of subgingival biofilms. *P. gingivalis* is known to adhere to *S. gordonii* through interaction of the short fimbrial antigen Mfa1 with a specific region of the streptococcal SspB polypeptide (designated BAR). In this regard, a recent study has designed the BAR peptide, a synthetic peptide comprising the BAR sequence, which has been reported to inhibit adherence of *P. gingivalis* to *S. gordonii*, thereby preventing the formation of *P. gingivalis*-associated biofilms [97].

### Natural sources

**Plant-derived antibacterial agents.** To overcome the alarming scarcity of new antibiotic classes, several recent studies have focused on finding new antibiotics from unexplored natural sources [98]. In this context, plants have proved to be a good new source for finding new antibacterial agents. This is not surprising, as plants are frequently exposed to bacterial infections and thus have developed various defense mechanisms to combat bacterial pathogens. Table 2 gives an overview of new antibiotics derived from plants that affect *P. gingivalis* biofilm formation.

The non-dialyzable material fraction of cranberry juice rich in proanthocyanidins and A-type cranberry proanthocyanidins extracted from cranberry juice concentrate were shown to exhibit activity against *P. gingivalis* biofilms [99,100]. The latter fraction was also found to affect adherence to oral epithelial cells negatively and have anti-inflammatory activities by inhibiting the secretion of interleukin-8 and chemokine ligand 5 [100]. Of

note, the activity of these A-type cranberry proanthocyanidin can be increased by combination therapy with Licochalcone A, a major chalcone in licorice root [109].

A number of prenylated flavonoids isolated from *Epimedium* species were reported to inhibit biofilm formation by *P. gingivalis* and to interfere with Rgp and Kgp gingipain activity [101].

Lacinartin derived from *Citrus* fruits and Tea catechin derived from *Camellia sinensis* have been demonstrated to inhibit biofilm formation of *P. gingivalis* biofilms and to desorb pre-formed biofilms [102,103]. Furthermore, Lacinartin negatively affected adherence to epithelial cells.

Extracted oils obtained from plants also possess activity against *P. gingivalis* biofilms. Indeed, essential oils extracted from medicinal and aromatic plants such as *Aloysia gratissima*, *Coriandrum sativum* L., *Muhlenbergia glomerata*, *Cyperus articulatus*, *Lippia sidoides*, and from shiitake have been reported to inhibit *P. gingivalis* biofilm formation [104,105]. Additionally, carvacrol, a monoterpene phenol present in the volatile oils of *Thymus vulgaris*, *Carum copticum*, and *oreganum* species, inhibits *P. gingivalis* biofilm formation on titanium implant material [106].

Recent studies revealed the antibiofilm effects of roselle calyx extract and capsaicin, which is the active compound of *Capsicum* plants (chili peppers) against *P. gingivalis* [107,108]. The latter also reduces the viability of pre-formed biofilms and has an inhibitory effects on both inflammatory cytokine secretion and *in vitro* osteoclastogenesis.

**Saccharides of marine origin.** In recent years, the marine environment has been explored as a source for finding new natural antibacterial agents. In this

**Table 2.** Overview of plant-derived compounds effective against *P. gingivalis* biofilm formation.

Plant-derived compound	Origin	Active concentration	Reference
Non-dialysable material from cranberry juice	<i>Vaccinium macrocarpon</i>	62.5 µg/mL	[99]
A-type cranberry proanthocyanidins	<i>Vaccinium macrocarpon</i>	50 µg/mL	[100]
Prenylated flavonoids	<i>Epimedium</i> species	1.25 µM	[101]
Lacinartin	<i>Citrus</i> fruits	50 µg/mL	[102]
Tea catechin epigallocatechin gallate	<i>Camellia sinensis</i> (tea plant)	10 µg/mL	[103]
Essential oils	Medicinal and aromatic plants ( <i>Aloysia gratissima</i> , <i>Coriandrum sativum</i> L., <i>Muhlenbergia glomerata</i> , <i>Cyperus articulatus</i> , and <i>Lippia sidoides</i> )	0.125–1 mg/mL	[104]
Essential oil	Shiitake mushroom ( <i>Lentinula edodes</i> )	0.97 µg/mL	[105]
Carvacrol	<i>Thymus vulgaris</i> , <i>Carum copticum</i> , and <i>oreganum</i> species	1%	[106]
Roselle calyx extract	<i>Hibiscus sabdariffa</i> L.	0.9 mg/mL	[107]
Capsaicin	<i>Capsicum</i> plants (chili peppers)	32 µg/mL	[108]

context, OligoG, which is an oligosaccharide derived from brown algae alginate, was found to reduce biofilm formation of *P. gingivalis* drastically [110]. Furthermore, treatment of titanium surfaces with triclosan combined with OligoG significantly decreases *P. gingivalis* attachment to titanium surfaces compared with treatment of the surfaces with triclosan alone.

Chitosan, which is a natural linear polysaccharide derived from chitin present in the exoskeleton of marine crustaceans, has also been reported to have antibiofilm activities against *P. gingivalis* [111].

### Sugar alcohols

Sugar alcohols are commonly used in place of sucrose as non-cariogenic sweeteners. However, little is known about their activity against periodontal bacteria [112]. A recent study reported that the sugar alcohol erythritol effectively inhibits *P. gingivalis* biofilm formation and reduces *P. gingivalis* accumulation onto *S. gordonii* substrata [113]. The authors concluded that erythritol acts via several pathways, including suppression of growth resulting from DNA and RNA depletion, attenuated extracellular matrix production, and alterations of dipeptide acquisition and amino acid metabolism.

### Other compounds

Screening of compound libraries has resulted in the identification of new antibacterial agents that show activity against *P. gingivalis*. We screened a compound library in search for new molecules that exhibit activity against the opportunistic pathogen *Pseudomonas aeruginosa* [114]. From this screening, a dichlorocarbazol derivative was identified as a new antibacterial agent with broad-spectrum activity, including activity against *P. gingivalis* biofilms. In another study, a library of small molecules based on 2-aminoimidazole and 2-aminobenzimidazole scaffolds was screened with the aim of identifying compounds that could inhibit co-colonization of *P. gingivalis* and *S. gordonii*. In this screening, three small molecules derived from oroidin and containing 2-aminoimidazole or 2-aminobenzimidazole moieties were identified. These compounds inhibit co-colonization by reducing expression of both *mfa1* and *fimA* fimbrial genes in *P. gingivalis* [115].

Drug repurposing has increasingly been applied over the last years as a strategy to uncover new antibiotics. This strategy has some advantages over *de novo* drug discovery, including known toxicological and pharmacological profiles, thereby accelerating the drug-development process significantly [116]. In this context, we recently screened a drug-repositioning library (NIH Clinical Collection) to identify new compounds that show activity against *P. gingivalis* [117]. The screen led to the discovery of three new molecules

that show antibiofilm activity against *P. gingivalis*: zafirlukast, an anti-asthmatic drug, toremifene, an anti-cancer drug, and N-(4-Hydroxyphenyl)arachidonamide (AM404), an active metabolite of paracetamol [117–119]. The anthelmintic drug oxantel, which is typically used for the treatment of intestinal worms, has also been proven to inhibit biofilm formation by *P. gingivalis* significantly by inhibition of fumarate reductase. Furthermore, oxantel is more effective than the conventional antibiotic metronidazole in inhibiting *P. gingivalis* biofilms [120]. In a follow-up study, it was demonstrated that oxantel can disrupt the development of polymicrobial biofilms composed of *P. gingivalis*, *Tannerella forsythia*, and *T. denticola* in a concentration-dependent manner [121].

### Antibacterial coatings

The coating of titanium surfaces with antibacterial agents has recently been explored as a new strategy for the prevention of peri-implant infections [122]. A number of studies have investigated the potential of antibacterial peptides to be used in coating applications. Indeed, coatings that are functionalized with GL13K (derived from the human salivary protein Parotid Secretory Protein [BPFA2]), histatin-5 (belonging to a family of peptides secreted by the major salivary glands), lactoferrin (generated by gastric pepsin cleavage of lactoferrins), and synthetic peptide Tet213 have been demonstrated to strongly reduce *P. gingivalis* biofilm formation [123–125].

The antibiofilm activity of titanium surfaces coated with silver has also been explored. As such, titanium surfaces coated with silver-hydroxyapatite/titania nanocomposites have been shown to act in both a bactericidal and anti-adhesive way against *P. gingivalis* [126]. In addition, the potential of silver- and gallium-doped phosphate-based glasses to inhibit growth of *P. gingivalis*–*S. gordonii* dual-species biofilms has been demonstrated [127]. Furthermore, a follow-up study showed that the gallium-doped phosphate-based glasses remain active against *P. gingivalis* under *in vivo* conditions [128].

In addition, bifunctional coatings with both antibacterial and pro-osteodifferentiation capabilities have been developed. Simvastatin is known to increase the osteogenic capability of mesenchymal stem cells, while metronidazole is an antimicrobial agent that has excellent activity against strict anaerobic bacteria. Integration of these drugs into a calcium phosphate coating for titanium surfaces prevents the growth of *P. gingivalis* and increases osteogenic cell differentiation [129].

### Concluding remarks

*P. gingivalis* is a notorious pathogen in the development of periodontitis and peri-implantitis. As these infections are biofilm-related, conventional antimicrobials often fail

to eradicate the entire biofilm, which results in chronic infections and necessitates surgical removal of infected areas. Thus, there exists a need for the development of new antibacterials to combat biofilm-related infections.

In recent years, a significant number of new compounds with antibiofilm activity against *P. gingivalis* have been identified. Unfortunately, to our knowledge, only one compound has progressed to clinical trials: the antibacterial peptide lactoferrin [94]. Different factors may explain the limited availability of new antibacterial drugs. For example, in spite of the promising results of the above-mentioned antibacterial peptides, there are still some challenges to their applications, such as potential toxicity, susceptibility to proteases, and high production costs [130]. As for the natural products interfering with *P. gingivalis* biofilm formation, limited information is currently present on their mode of action and their cytotoxicity. In addition, the active concentrations of some plant-derived compounds are up to 1,000 times higher than conventional antibiotics, indicating limited antibacterial activity [83,84,87]. Regarding the surface coating strategies to prevent biofilm formation on implants, there still exists a great discrepancy between the suggested strategies and their clinical applications [131]. Furthermore, potential limitations of these coatings such as toxicity and hampered antibacterial activity under *in vivo* conditions should be tackled in future studies [131,132].

Thus, further mode-of-action studies, comprehensive toxicity analyses, and *in vivo* tests will be necessary to reveal fully the potential of newly discovered antibacterial agents to be used in the treatment of oral infections. In addition, a broader knowledge of the regulatory and molecular mechanisms behind *P. gingivalis* biofilm formation may further accelerate the development of future strategies for treatment of *P. gingivalis*-associated infections.

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